Vitamin A and Carotene Stability in Feeds Containing Antioxidant-Treated Animal Fats

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Experimental storage tests were conducted on the effect of antioxidant-treated animal fat in increasing the stability of vitamin A, carotene, and the fat in commercial poultry feeds. Antioxidants tested were 2-(and 3-)*tert*-butyl-4-hydroxyanisole (BHA); 6-ethoxy-2,2,4-trimethyl-1,2-dihydroquinoline (Santoquin); BHA plus 2,5-di-*tert*-amylhydroquinone (DAH); 2,6-di-*tert*-butyl-p-cresol (BHT); BHA plus BHT; and *N,N'*-diphenyl-p-phenylenediamine (DPPD). All the antioxidant treatments were effective in increasing the vitamin A, carotene, and fat stability over that observed when nonstabilized fat or no fat was added. Santoquin was shown to be the most effective of the antioxidants tested at the 0.02% level, followed closely by DPPD, BHT, and BHA plus BHT.

INCREASED USE OF ANIMAL FATS in commercial poultry feeds has resulted from recent studies on this effect (*17, 18, 20, 23*). These fats usually are stabilized against rancidity development by the addition of appropriate antioxidants. The effects of antioxidant stabilization of these animal fats on the stability of other nutrients in the feeds, especially the fat-soluble vitamins, have stimulated considerable interest.

In recent studies on the stability of vitamin A in mixed feeds containing antioxidant-treated or untreated animal fats, it was shown that the added stabilized fat increased the stability of the vitamin A in the feed during storage (*19*). A number of other investigators also have studied antioxidant stabilization of the carotene in alfalfa meals and have shown that antioxidant treatments will increase the stability of the carotene (*5, 11, 21*).

In the present study the effects of various antioxidants on the stability of vitamin A and carotene were determined in mixed poultry feeds during storage at 100° F.

A number of antioxidants, including some which have been approved for use in foods and feeds, were selected for these tests on the basis of previous studies on their effectiveness in stabilizing animal fats, vegetable fats, or carotene in alfalfa. In addition to vitamin A and carotene stability, the peroxide values of the fats in the feed and organoleptic evaluations of the feeds for rancidity were determined during the 18-week storage period.

Bioassays for vitamin A, or carotene, were made on some of the stored samples in the second series in order to test the validity of the chemical assays.

Experimental

Four different poultry-type feeds were used in each of two series of experiments. The compositions of diets 1 and 2 are shown in Table I. Diet 3 is the same as diet 2, with the exception of the fish meal which was solvent-extracted to remove the fish oils before addition to diet 3. The fish meal contained approximately 10% residual oils before extraction and less than 1% after extraction with petroleum ether (Skellysolve B). Diet 4 is the same as diet 2, except that synthetic vitamin A palmitate (Myvax, 200,000 U. S. P. units per gram) replaced the dehydrated alfalfa meal as the primary source of vitamin A activity.

White corn was used in all the diets except diet 1 in order to limit the source of carotene or vitamin A to one major ingredient in each diet. Diet 1 is a practical type diet and is similar to the ration used in previous studies with chicks (*17*).

A blend of choice white grease–choice tallow (1 to 1) was used as the animal

Table I. Composition of Diet 1 (Yellow Corn–Fish Oil) and Diet 2[a] (White Corn–Alfalfa)

Ingredient	*Diet 1, %*	*Diet 2, %*
White corn (ground)	...	62.73
Yellow corn (ground)	62.55	...
Soybean grits (solvent-extracted)	22.30	19.72
Meat scrap	5.00	4.00
Fish meal (menhaden)	4.00	8.00
Corn gluten meal	2.00	...
Alfalfa leaf meal (dehydrated)	2.00	3.50
Butyl fermentation solubles (BY-500)	1.00	1.00
Fish oil (2250 U.S.P. units A; 400 A.O.A.C. units D gram)	0.20	[c] ...
Iodized salt	0.50	0.50
Choline Cl	0.20	0.25
Aurofac[b]	0.25	0.20
	100.00	100.00
Supplements	*Mg./Kg. Ration*	*Mg./Kg. Ration*
$MnCl_2.4H_2O$	320	320
Niacin	20	20

[a] Diet 3 same as diet 2, except fish meal was replaced with solvent-extracted fish meal. Diet 4 same as Diet 2 except that alfalfa meal was replaced with synthetic vitamin A palmitate (Myvax) and suitable adjustment of white corn and soybean grits was made.
[b] Aureomycin (1.8 grams per pound) + vitamin B_{12} (1.8 γ per pound) supplement.
[c] 0.1% vitamin D_3 supplement (1500 I.C. units per gram) added.

Table II. Antioxidant Treatments Studied during Storage of Feeds at 100° F.

Treatment Number	Treatments[a]
	Series I
1	No fat added
2	+6% unstabilized fat
3	+6% stabilized fat (0.02% BHA)
4	+6% stabilized fat (0.02% Santoquin)
5	+6% stabilized fat (0.01% BHA + 0.01% DAH)
6	+6% stabilized fat (0.02% BHT)
7	+6% stabilized fat (0.01% BHA + 0.01% BHT)
8	+6% stabilized fat (0.02% DPPD)
	Series II
1	No fat added
2	+6% unstabilized fat
3	+6% stabilized fat (0.02% BHA)
4	+6% stabilized fat (0.02% Santoquin)
5	(Not included in series II)
6	+6% stabilized fat (0.02% BHT)
7	+6% stabilized fat (0.01% BHA + 0.01% BHT)
8	+6% stabilized fat (0.02% DPPD)
9	+6% stabilized fat (0.10% Santoquin)
10	+6% stabilized fat (0.10% BHT)
11	+6% stabilized fat (0.10% DPPD)

[a] Citric acid added at 0.01% to fat for all antioxidant treatments.

fat added to the basal diets in both series I and II. The same level of animal fat (6%) was added to all the diets containing added fat. The total fat levels are uniform in each series, as only minor variations occurred in the fat content of the basal rations used.

The antioxidants used were 2-(and 3-) *tert*-butyl-4-hydroxyanisole (BHA); 6-ethoxy - 2,2,4 - trimethyl - 1,2 - dihydroquinoline (Santoquin); 2,6-di-*tert*-butyl-*p*-cresol (BHT); (BHA) plus 2, 5-di-*tert*-amylhydroquinone (DAH); *N,N'*-diphenyl-*p*-phenylenediamine (DPPD); and BHA plus BHT. The total antioxidant concentration used was 0.02% of the added animal fat and 0.01% citric acid was added in all cases.

The storage tests were divided into two separate series. The second series duplicated seven of the eight treatments studied in the first series. In addition, BHT, DPPD, and Santoquin were tested at a level of 0.1% of the added animal fat in storage tests in series II. The experimental treatments for each diet studied in series I and series II are shown in Table II.

All the antioxidants were added as 10% ethyl alcohol solutions to the melted fat at 80° C. with mechanical stirring, except for DPPD which was added directly to the heated fat with prolonged mechanical stirring.

The active oxygen method (AOM) stability values for the treated and untreated animal fats used in series I and II and the titer, color, free fatty acid content, and peroxide value of the untreated fats are shown in Table III.

Seven individual 300-gram samples of each dietary treatment were taken from a composite lot of the mixed feed and were stored in 1-pound glassine-lined paper bags at 100° F. in a constant temperature room. Individual samples were used to permit removal of one bag at each storage period for the analyses. The contents of each sample bag were thoroughly mixed prior to the analysis.

The peroxide value of the fat in the feed, vitamin A and carotene determinations, and organoleptic evaluations for rancidity of the stored samples were made at 0-, 2-, 4-, 6-, 10-, 14- and 18-week intervals during storage.

Peroxide value determinations of the fat in the feeds were made according to the American Oil Chemists' Society tentative methods (*1*).

It was difficult to detect rancidity development organoleptically in the feeds containing no added fat because of the low fat content. In all cases, when one or more of the individuals examining the samples were undecided as to whether the feed was rancid or not, the designation (±) was used to indicate questionable rancidity. Since an individual bag of feed was examined and used at each storage period, variables due to previous handling at earlier storage periods were avoided.

Carotene was determined by the Association of Official Agricultural Chemists' (AOAC) chromatographic method of analysis (*3*) in all samples except those containing DPPD. Beauchene and coworkers have indicated that the presence of DPPD interferes with the AOAC method for analysis for carotene (*4*); therefore a modification, using tricalcium phosphate as the adsorbent, as reported by Mitchell and Silker (*12*) was used for those samples containing DPPD.

The chromatographic method for the determination of vitamin A in mixed feeds as reported by Schaeffer (*16*) was used in the analysis for vitamin A. Before this work was completed, it was reported that DPPD interferes with the vitamin A determinations, causing high values (*14*); however, at the levels of DPPD used in the present experiments, it is believed that the interference is negligible. Separate investigations indicated that the above methods were satisfactory for carotene and vitamin A determinations in these studies.

In the storage studies of series II (Table II) the vitamin A biopotencies of samples containing no added fat, 6% added unstabilized fat, and 6% added fat stabilized with 0.02% Santoquin, 0.02% DPPD, or 0.02% BHT were determined by chick growth (*10*), chick liver storage (*2*), and rat growth bioassay methods (*22*). The bioassay values were determined at the

Table III. Analyses and Stability Values of Fats Used in Carotene–Vitamin A Stability Studies

Analytical Values for Unstabilized Fat	Series I	Series II
P. v.[a]	3.0	3.5
Titer	40.7° C.	40.5° C.
Color (FAC)	11	9
Free fatty acid, %	2.60	2.70
Stability Data		
Stabilizer	*AOM, Hr.*	*AOM, Hr.*
None	4	3
0.02% BHA + 0.01% citric acid	110	139
0.02% Santoquin + 0.01% citric acid	43.5	49
0.02% BHT + 0.01% citric acid	72	47
0.01% BHT + 0.01% BHA + 0.01% citric acid	104	102
0.01% DAH + 0.01% BHA + 0.01% citric acid	79	...
0.02% DPPD + 0.01% citric acid	>257	>254
0.10% BHT + 0.01% citric acid	...	165
0.10% Santoquin + 0.01% citric acid	...	>271
0.10% DPPD + 0.01% citric acid	...	>255

[a] Peroxide value. Meq. of oxygen per kilogram of fat.

start of the storage test and after approximately 50% of the vitamin A activity had been destroyed on storage as indicated by the chemical analyses.

The rat growth bioassay procedure was essentially that described in the U. S. Pharmacopoeia XIV, however, since the test supplements were animal feeds, individual dosing consisted of feeding each rat a weighed amount of supplement twice weekly with suitable precaution to recover and refeed spillage. The supplement feeds were stored at 0° F. between dosage intervals and freshly mixed each week during the bioassay period. The test diet supplements were equally diluted with the depletion ration in order to facilitate the supplementation of vitamin A within the dosage range and to avoid possible variation in response other than that for vitamin A activity.

The chick growth bioassay was performed using 20-day-old White Rock cockerels in each group. After 7 to 10 days on a vitamin A depletion diet, the vitamin A standard and supplements (0.25 to 1.0 U.S.P unit per gram of feed) were incorporated into the depletion diet. All the feed supplements were added to the depletion diet at the same concentrations. The supplemented feeds were stored at 0° F. between feedings and freshly mixed each week during the bioassay. The chicks were fed the supplements ad libitum for 4 weeks and individual weights recorded each week.

At the termination of the chick growth bioassay, the levels of vitamin A standards and supplements were increased to levels suitable for liver storage. The chicks were fed the increased supplementations (2.0 to 6.0 U.S.P. units per gram of feed) for 1 week, after which 10 chicks from each group were sacrificed and the livers removed and analyzed for vitamin A by the Carr-Price reaction. The vitamin A values were determined for 10 livers in each group by pooling two individual livers for each analysis.

Three levels of standard dosages were used in each bioassay (both rats and chicks) for determining the standard response curve and one level of each of the feeds.

The samples for the bioassay at 50% destruction of vitamin A activity were stored at 100° F. in 50-pound glassine-lined paper bags until the chemical values indicated approximately one half of the vitamin A activity had been destroyed. These samples were then stored at 0° F. until bioassayed.

Results and Discussion

The results of the series I storage tests for vitamin A and carotene stability as determined chemically are shown graphi-

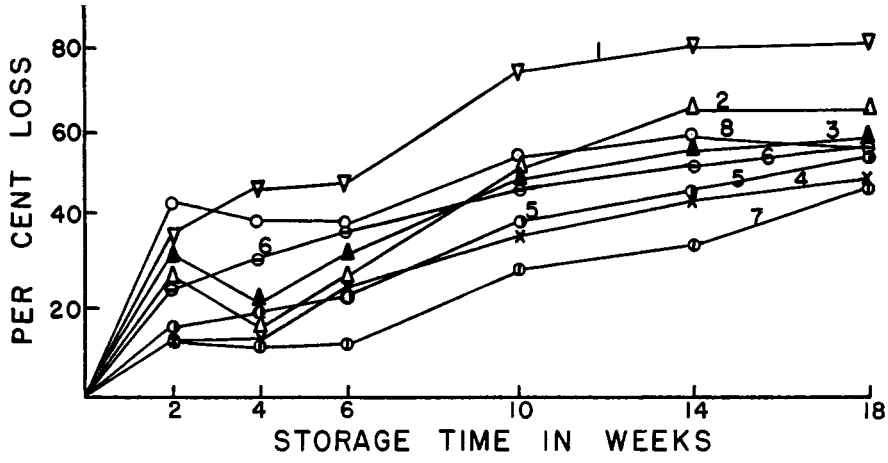

Figure 1. Vitamin A loss (%) during storage at 100° F. for 18 weeks in series I tests (Diet 1. Fish liver oil, alfalfa, yellow corn)

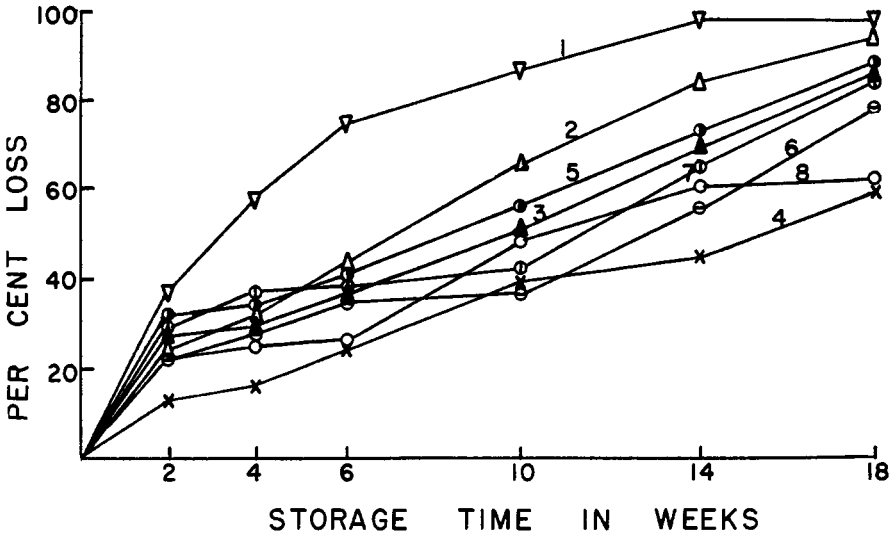

Figure 2. Carotene loss (%) during storage at 100° F. for 18 weeks in series I tests (Diet 1. Fish liver oil, alfalfa, yellow corn)

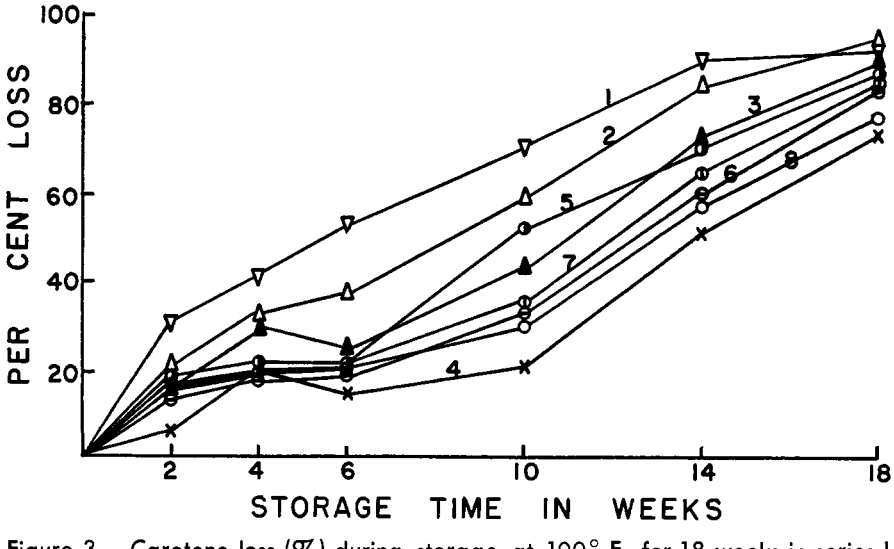

Figure 3. Carotene loss (%) during storage at 100° F. for 18 weeks in series I tests (Diet 2. Alfalfa, white corn)

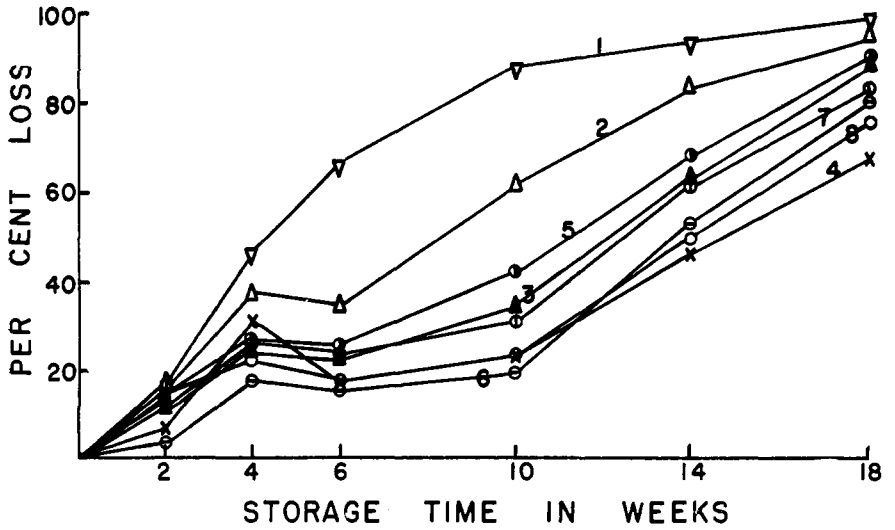

Figure 4. Carotene loss (%) during storage at 100° F. for 18 weeks in series I tests (Diet 3. Alfalfa, white corn, extracted fish meal)

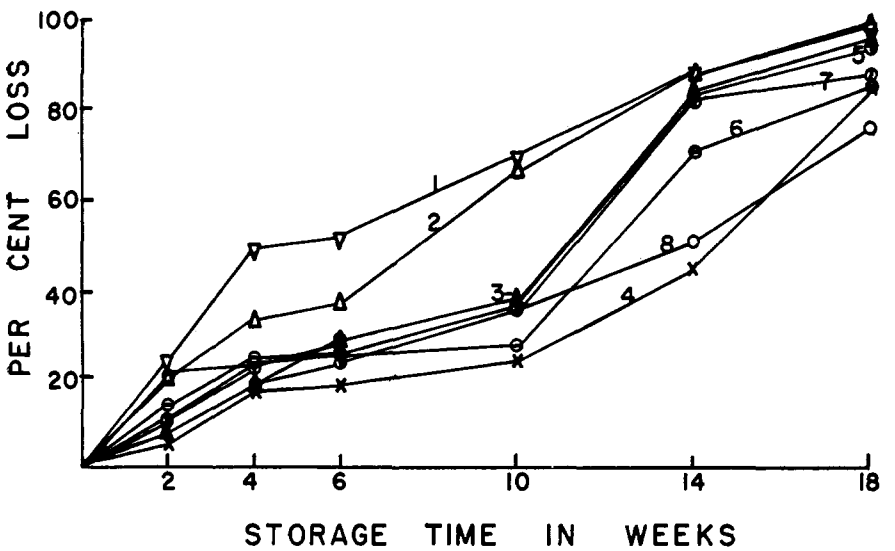

Figure 5. Vitamin A loss (%) during storage at 100° F. for 18 weeks in series I tests (Diet 4. Synthetic vitamin A, white corn)

cally in Figures 1 through 5 and the specific results for diet 2 and diet 4 are shown in Tables IV and V. These results are typical of those observed for the other diets used.

Organoleptic evaluations of the feed indicated that rancidity of the fat had developed in the control feeds (no added fat) by the fourth week of the storage period, while the diets with nonstabilized fat added became rancid by the sixth through the tenth weeks of storage. This stabilizing effect of the added fat was essentially eliminated by the end of the storage period. Similar results on the stability of carotene in alfalfa meal with added oil or fat have also been reported by Mitchell and Silker (*13*) and Bickoff and coworkers (*6*). None of the antioxidant-treated samples was considered rancid until the fourteenth week of storage. The corresponding peroxide values and rate of loss of vitamin A or carotene correlated well with the rancidity development (Tables IV and V).

No striking effects were noted that could be attributed to the use of fish meal from which the fat was removed (diet 2 compared to diet 3). The carotene stability appeared to be slightly less when the fish meal was extracted and no fat added; however, all antioxidant effects were similar to those observed for diet 2.

The effectiveness of specific antioxidant treatments was similar with each of the diets used. On an over-all basis, BHT, BHA plus BHT, Santoquin, and DPPD appeared to be more effective than BHA or BHA plus DAH. These results do not correlate well with the AOM stability of the stabilized fats before addition to the feed (Table III). The combination of BHA plus BHT was more effective in stabilizing vitamin A in diet 1 than either BHA or BHT alone. However, BHA plus BHT was not as effective as BHT in stabilizing the vitamin A in diet 4.

An additional dietary treatment was tested in which the alfalfa was dehydrated by deep frying in hot fat containing the specific antioxidant treatment to be tested. Because of the low carotene content and extremely high fat retention of the alfalfa, a sufficient amount of carotene could not be incorporated into the diet to compare with the other test diets; however, all storage samples became rancid and carotene content negligible by the fourth storage period (6 weeks). No significant differences were noted between treatments and these tests were not repeated in series II.

The chemical and organoleptic data obtained in the series II tests for diets 2 and 4 are shown in Tables VI and VII. The data for the vitamin A, carotene, and fat stability of all the antioxidant treatments of diets 1 and 3 were in excellent agreement with those observed for series I.

The stability of vitamin A and carotene was the lowest for the diets containing no added fat. The addition of unstabilized animal fat increased the stability of the vitamin A or carotene over that of the diets with no fat added. Again, no appreciable differences were noted that could be attributable to the use of fat-extracted fish meal.

All of the antioxidants tested were effective in increasing the stability of the vitamin A, carotene, and fat in the diets; however, the over-all stabilities were slightly better in series II than in series I. Santoquin, BHT, BHA plus BHT, and DPPD again were the most effective of the antioxidants tested.

In the storage tests of series II the effect of added fat stabilized with 0.10% BHT, 0.10% Santoquin, or 1.10% DPPD was also studied in diets 2 and 4. The addition of these antioxidants to the fat at the 0.10% level increased the AOM stability of the fat considerably above the AOM stabilities observed when 0.02% levels were added (Table III) and also increased the stability of the vitamin A, carotene, and fat in diets 2 and 4 over that observed at the 0.02% levels of antioxidant (Tables VI and VII). This increased stability was primarily observed during the latter stages of the storage period (14 to 18 weeks).

Table VIII gives a summarization of the vitamin A and carotene stabilities of both series I and series II tests. The percentages of vitamin A and carotene losses are given for the 6th-, 10th-, and 14th-week storage periods, since

concurrent rancidity development and increased rate of destruction of vitamin A or carotene occurred at approximately 4 to 6 weeks in the control diets (no fat added), at 6 to 10 weeks in the diets containing added unstabilized fat, and at 10 to 14 weeks in most of the stabilized fat diets in both series I and II. The average percentage loss of vitamin A and carotene for duplicate treatments of series I and II is also summarized in Table VIII.

Santoquin was shown to be the most effective of the antioxidants or antioxidant combinations tested at the 0.02% level, followed closely by DPPD, BHT, and BHA plus BHT based on the stability of the fat, vitamin A, and carotene throughout the 18-week storage period. BHA, or BHA plus BHT, showed comparable stabilization of the vitamin A in diet 4 (synthetic vitamin A) in series II, whereas in series I, BHT appeared to be more effective than BHA plus BHT. The AOM stability values of the animal fat stabilized with antioxidants at the 0.02% level did not correlate with the stability of the fat or vitamin A and carotene after addition of the fat to the feed. For example, Santoquin treatment resulted in the lowest stability of the fat, but the greatest stability of the fat, carotene, and vitamin A in the mixed feed.

The stability of the fat in the diets correlates very well with the carotene and vitamin A stabilities as determined by peroxide values and organoleptic evaluation. The increases in peroxide

Table IV. Stability Data for Diet 2

Analysis Made	Storage Period, Weeks	Treatment No.[a] 1	2	3	4	5	6	7	8
Fat content, %		3.95	9.90	9.85	10.00	10.05	10.90	10.10	9.90
Carotene content, USP units per gram	0	11.8	12.6	12.5	13.1	12.4	12.9	12.6	14.1
	2	8.1	9.9	10.5	12.4	10.2	12.9	10.4	11.7
	4	6.9	8.4	9.8	10.3	9.7	10.4	10.0	11.1
	6	5.6	7.9	9.2	11.2	9.8	10.4	10.0	11.2
	10	3.6	5.2	7.1	10.3	6.8	8.5	7.5	9.8
	14	1.3	2.1	3.4	6.5	3.7	5.2	4.8	6.0
	18	1.1	0.7	1.4	3.7	1.7	1.0	2.0	3.3
Peroxide value of fat in feed	0	20.0	7.3	7.9	8.0	6.1	6.7	5.8	5.1
	2	21.4	9.0	8.3	8.2	5.2	3.4	4.1	4.1
	4	19.7	9.5	9.0	7.9	7.7	7.2	7.4	8.7
	6	18.2	11.4	6.2	4.9	6.0	4.6	5.1	5.8
	10	22.6	22.4	13.6	7.3	12.4	5.3	8.5	6.4
	14	18.3	33.6	24.4	13.8	21.2	21.8	22.2	10.8
	18	26.6	61.0	48.0	34.0	36.3	32.5	37.0	14.0
Organoleptic evalutions of feed[b]	0	±	−	−	−	−	−	−	−
	2	+	−	−	−	−	−	−	−
	4	+	−	−	−	−	−	−	−
	6	+	±	−	−	−	−	−	−
	10	+	+	±	−	±	−	±	−
	14	±	+	±	±	±	±	±	−
	18	±	+	+	+	+	+	+	−

[a] See Table II.
[b] −. Not rancid. ±. Questionable. +. Rancid.

Table V. Stability Data for Diet 4

(Synthetic vitamin A, series I)

Analysis Made	Storage Period, Weeks	Treatment No.[a] 1	2	3	4	5	6	7	8
Fat content, %		4.10	10.00	10.05	9.80	9.70	10.35	10.20	10.29
Vitamin A, content, USP units per gram	0	15.5	14.2	14.7	14.6	13.7	14.5	15.4	16.1
	2	11.8	11.3	13.3	13.8	12.5	12.4	13.6	12.8
	4	8.0	9.6	11.9	12.1	11.9	11.1	11.9	12.3
	6	7.6	8.9	10.4	11.8	10.4	10.7	10.9	11.7
	10	4.9	4.8	8.9	11.2	8.5	10.4	9.8	10.1
	14	1.8	1.9	2.1	8.1	2.0	4.3	2.6	8.0
	18	0.5	0.6	0.7	2.3	0.6	2.3	2.0	4.0
Peroxide value of fat in feed	0	19.7	7.2	7.0	7.3	8.4	7.7	7.3	6.3
	2	15.8	6.2	5.7	4.8	4.2	3.6	4.2	3.6
	4	24.6	20.9	6.6	3.2	10.7	4.2	4.5	5.3
	6	26.2	29.0	9.3	6.0	14.3	4.5	5.1	4.3
	10	35.5	60.5	31.2	8.4	51.0	14.5	22.8	7.4
	14	32.1	102.0	57.0	35.0	89.4	72.0	68.0	12.2
	18	35.5	109.0	104.0	83.0	114.0	122.0	110.0	21.6
Organoleptic evaluations of feed	0	±	−	−	−	−	−	−	−
	2	±	−	−	−	−	−	−	−
	4	+	+	−	−	±	−	−	−
	6	+	+	−	−	±	−	−	−
	10	+	+	±	−	+	±	±	±
	14	+	+	+	±	+	+	+	±
	18	+	+	+	+	+	+	+	±

[a] See Table II.

values of the stored feeds and organoleptic detection of rancidity were accompanied by a corresponding increase in vitamin A or carotene destruction. Buxton (*7*), Dyme and coworkers (*9*), and Sandell (*15*) have indicated that vitamin A destruction generally parallels the rise in peroxide value of the vitamin carrier.

The results of the chick growth, rat growth, and chick liver storage in series II indicated the chemical values were valid measures of the biological activity of the vitamin A or carotene contained in these feeds (0.6 γ of carotene was assumed equal to 1 USP unit of vitamin A in both the rat and chick). No apparent differences were noted between the chemical or biological values of the vitamin A activity in either the fresh (0 week storage), or stored (50% destroyed vitamin A or carotene) samples. The general consistency of response per unit of vitamin A activity for the different treatments indicated little or no effects due to the antioxidant treatments at the levels tested.

The values at 0-week storage for diet 1 were considerably higher than the chemical values; however, this diet consists of 62% yellow corn which may have vitamin A active pigments other than carotene (*8*). The bioassay values of diet 1 after storage did not show this increased vitamin A activity over that indicated by the chemical values.

These results show that several antioxidants and antioxidant combinations are effective in stabilizing the added animal fat, vitamin A, and carotene in poultry-type feeds during storage. It is significant that the addition of non-

Table VI. Stability Data for Diet 2

(Alfalfa, series II)

Analysis Made	Storage Period, Weeks	Treatment No.[a] 1	2	3	4	5	7	8	9	10	11
Fat content, %		3.63	8.56	9.03	9.76	9.07	9.42	8.73	9.36	9.53	9.36
Carotene content, USP units per gram	0	9.0	9.5	10.0	9.7	9.5	10.0	9.7	10.0	9.7	9.7
	2	6.9	8.0	8.8	9.0	8.7	8.5	9.0	9.8	9.3	9.2
	4	4.7	7.5	7.7	8.4	7.9	7.9	8.4	9.1	8.7	8.9
	6	1.7	4.9	6.5	8.0	6.8	6.8	8.2	8.7	7.5	8.7
	10	1.0	1.4	6.0	7.9	6.7	6.5	8.1	8.6	8.1	8.8
	14	0.5	0.5	3.5	5.9	5.3	4.1	5.3	7.2	6.3	6.3
	18	<0.5	<0.5	2.5	3.4	2.6	2.2	3.5	6.6	5.0	4.7
Peroxide value of fat in feed	0	23.6	9.6	10.5	10.1	10.7	9.8	8.9	9.6	10.0	8.0
	2	23.4	9.7	7.0	7.3	7.0	7.3	7.0	6.6	6.8	6.0
	4	26.7	15.2	8.2	6.3	8.9	7.7	8.0	6.3	5.2	5.9
	6	35.6	33.0	7.5	6.3	7.7	6.4	7.7	4.9	4.8	1.8
	10	57.0	111.0	9.4	11.2	8.5	6.9	10.1	6.0	6.8	5.2
	14	51.5	172.0	16.7	8.5	9.9	15.3	9.9	5.5	6.6	5.6
	18	22.3	102.0	25.8	5.3	10.9	23.8	7.6	5.5	5.5	7.5
Organoleptic evaluations of feed	0	−	−	−	−	−	−	−	−	−	−
	2	−	−	−	−	−	−	−	−	−	−
	4	+	+	−	−	−	−	−	−	−	−
	6	+	+	−	−	−	−	−	−	−	−
	10	+	+	−	−	−	−	−	−	−	−
	14	±	+	−	−	−	−	−	−	−	−
	18	±	+	+	−	−	±	−	−	−	−

[a] See Table II.

Table VII. Stability Data for Diet 4

(Synthetic vitamin A, series II)

Analysis Made	Storage Period, Weeks	Treatment No.[a] 1	2	3	4	6	7	8	9	10	11
Fat content, %		4.02	9.06	9.34	9.12	9.50	9.86	9.40	9.17	9.67	9.83
Carotene content, USP units per gram	0	14.5	14.8	14.2	14.5	14.0	14.3	14.5	14.3	14.5	14.8
	2	13.2	14.3	14.0	14.3	13.7	14.0	14.3	14.0	14.3	14.5
	4	6.3	10.7	13.5	13.0	11.3	13.0	14.1	13.2	13.5	13.2
	6	3.2	7.1	11.3	11.9	10.8	10.4	11.9	11.6	12.4	11.9
	10	2.4	2.3	10.2	11.7	10.5	9.6	11.9	11.2	11.9	11.9
	14	0.6	1.2	3.0	9.8	4.7	7.2	8.7	9.4	9.4	9.4
	18	<0.5	<0.5	1.7	6.4	5.5	5.1	6.0	9.0	7.5	8.1
Peroxide value of fat in feed	0	29.0	9.6	8.9	9.4	10.3	8.8	8.9	8.9	8.9	7.5
	2	27.5	8.9	4.5	4.9	4.7	4.6	4.7	3.9	3.6	3.2
	4	68.0	35.2	3.5	3.0	3.3	3.0	3.7	3.5	3.3	3.0
	6	84.0	450.0	4.7	4.3	3.8	3.6	6.9	2.0	3.8	2.2
	10	39.3	165.0	17.7	2.5	13.8	2.5	9.6	0.8	2.8	0.8
	14	26.9	48.0	123.0	17.4	128.5	14.7	30.0	5.9	2.6	4.0
	18	27.2	22.9	83.1	32.0	76.5	41.6	62.5	3.4	2.5	2.6
Organoleptic evaluations of feed	0	±	−	−	−	−	−	−	−	−	−
	2	±	−	−	−	−	−	−	−	−	−
	4	+	+	−	−	−	−	−	−	−	−
	6	+	+	−	−	−	−	−	−	−	−
	10	+	+	±	−	−	−	−	−	−	−
	14	±	+	+	−	+	−	−	−	−	−
	18	±	+	+	−	+	±	+	−	−	−

[a] See Table II.

stabilized fat decreases the losses of vitamin A and carotene during the early phases of the storage period in this and other studies (*6*, *13*). As the stability of animal fats, vitamin A, and carotene in the ration does not always correlate with the stability of the fat prior to addition to the ration as measured by the AOM test, it is clear that the stability of a complex system such as a feedstuff cannot be predicted with certainty by stability tests on the fat constituent alone. This does not imply that such fat-stability tests are of no value in predicting stability of fat or vitamin A in mixed feeds, but implies a recognition of their limitations.

The selection of an antioxidant for any specific end use depends on a number of facts, which include: effectiveness in stabilizing the fat, price of the antioxidant or antioxidant combination, effect on the color, utility for alternative uses of the stabilized product or other characteristics of the treated product, and status of approval for use by the Food and Drug Administration or other appropriate governmental agencies. The present study was designed to obtain data on the first factor mentioned.

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Table VIII. Summary of Vitamin A and Carotene Losses at 6, 10, and 14 Weeks' Storage

Diet	Series	Treatments[a] 1	2	3	4	5	6	7	8
					6 Weeks, Carotene Loss, %				
1	I	74	43	39	24	41	34	38	25
	II	54	33	35	17	..	12	30	11
2	I	53	37	27	15	21	19	21	21
	II	82	49	35	18	..	29	32	15
3	I	66	35	22	16	24	16	24	19
	II	85	51	25	12	..	17	16	9
Average		69	41	31	17	..	21	27	17
					6 Weeks, Vitamin A Loss, %				
1	I	48	27	32	26	24	37	12	41
	II	32	7	16	5	..	8	16	23
4	I	51	38	29	19	24	26	29	28
	II	78	52	21	18	..	23	27	18
Average		52	31	25	17	..	23	21	28
					10 Weeks, Carotene Loss, %				
1	I	86	66	50	39	56	37	42	49
	II	78	46	42	18	..	23	40	19
2	I	70	59	43	21	53	34	40	31
	II	89	87	40	19	..	30	35	16
3	I	86	62	35	24	43	20	31	24
	II	92	89	37	17	..	21	26	13
Average		84	68	41	23	..	28	36	25
					10 Weeks, Vitamin A Loss, %				
1	I	73	52	50	36	41	48	29	23
	II	43	37	40	5	..	8	16	17
4	I	69	67	40	23	38	28	36	37
	II	83	85	28	19	..	25	33	18
Average		67	60	40	21	..	27	29	24
					14 Weeks, Carotene Loss, %				
1	I	97	85	70	44	72	56	64	60
	II	91	83	86	54	..	62	81	43
2	I	89	84	73	51	71	60	62	65
	II	95	94	65	39	..	45	59	45
3	I	93	82	64	45	66	55	60	50
	II	95	95	74	46	..	57	67	55
Average		93	87	72	47	..	56	66	53
					14 Weeks, Vitamin A Loss, %				
1	I	80	64	56	44	47	55	34	60
	II	95	95	95	30	..	33	95	36
4	I	89	87	79	45	86	71	83	51
	II	96	92	79	32	..	66	49	40
Average		90	85	77	38	..	56	65	47

[a] See Table II.

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