Effect of Added Animal Fats and Antioxidant on Stability of Xanthophyll Concentrates in Mixed Feeds

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The stability of carotenoids in four commercial concentrates was evaluated. The carotenoids in two of the four concentrates studies were very unstable. During commercial preparation, one of the less stable concentrates had undergone a saponification treatment which probably removed natural antioxidants. Generally, addition of the concentrates to mixed feeds gave better carotenoid stability than when the materials were stored as such in open vials. The pigments of a lettuce meal concentrate were slightly less stable in a mixed feed than when stored alone. It was demonstrated that the addition of animal fats or an antioxidant, 6-ethoxy-1,2-dihydro-2,2,4-trimethylquinoline, improved the retention of carotenoids in mixed feeds. Use of the two together was more effective than either one used separately. Complete stabilization of the xanthophyll concentrates was achieved by storage in filled, sealed containers.

INCREASING AMOUNTS OF XANTHOPHYLLS are being employed in poultry feeds to impart a desirable yellow color to the skin and shanks of broilers and to give uniform yolk color in eggs. The stability of these pigments in mixed feeds is important because of the considerable time that often elapses between formulation of the feed and consumption by the bird. Wall and Kelley (*8*) reported that carotene in chick mash is destroyed rapidly when stored at room temperatures. Recently, Davies and Worden (*4*) found that cereal ingredients, mineral salts, and particle size affect the stability of vitamin A and carotene in poultry feed. Because carotene and xanthophyll are closely related chemically, stability of xanthophyll might be expected to resemble that of the carotene.

Earlier studies have shown that antioxidants (*3, 5*) and animal fats or vegetable oils (*2, 6*) are effective in stabilizing carotene in alfalfa meal. These results prompted the present investigation.

Commercial xanthophyll concentrates were combined with portions of a mixed feed. To certain of these fortified feeds, either animal fat or an antioxidant or both were added as stabilizers. Because the analytical method for xanthophyll can be used for the determination of carotene (*1*), the storage stability of the carotene present in the xanthophyll concentrates was also determined. For comparison, a commercial alfalfa meal was stored under comparable conditions.

Table I. Storage Stability of Commercial Xanthophyll Preparations

Sample and Source	Antioxidant Added, %	Initial Pigment Content, P.P.M.		Retention after 4 Months at 25° C., %	
		Xanthophyll	Carotene	Xanthophyll	Carotene
Concentrate A, alfalfa (saponified)	0	3,370	2635	0[a]	0[a]
	0.15	3,325	2568	0[a]	0[a]
Concentrate B, corn gluten	0	2,630	248	0[a]	0[a]
	0.15	2,963	291	0[a]	0[a]
Concentrate C, alfalfa	0	17,825	2040	31	36
	0.15	17,525	2075	42	73
Concentrate D, lettuce	0	4,443	1890	65	56
	0.15	4,680	1975	82	86
Concentrate A,[b] alfalfa	0	3,348	2601	99	100
	0.15	3,325	2568	100	100

[a] Two months at 25° C.
[b] Sealed containers.

Experimental Methods

Xanthophyll Concentrates Four commercial xanthophyll concentrates were studied; two were from dehydrated alfalfa meal, one from lettuce meal, and one from corn gluten meal (Table I). One of the concentrates from alfalfa was known to have undergone a saponification treatment to remove chlorophyll during manufacture. This material and the one from corn were thick, red oils. The unsaponified concentrates from alfalfa and lettuce meal were both thick, dark-green, waxlike products.

Commercial Mixed Feeds Two commercial mixed feeds were employed in this study. They were standard broiler mashes containing the following ingredients: ground corn, soybean oil meal, meat scraps, fish meal, dehydrated alfalfa meal, riboflavin supplement, D activated animal sterol (source of vitamin D_3), vitamin B_{12}, antibiotic supplements, methionine, niacin, calcium pantothenate, and eight minerals. Initially, broiler feed 1 contained 25 p.p.m. of xanthophyll and 13 p.p.m. of carotene, while broiler feed 2 contained 4 p.p.m. of xanthophyll and 3 p.p.m. of carotene.

Alfalfa Meal A high-quality commercially prepared alfalfa meal which contained initially 550 p.p.m. of xanthophyll and 270 p.p.m. of carotene was employed for comparison with the xanthophyll concentrates.

Stabilizers The antioxidant used was 6 - ethoxy - 1,2 - dihydro-2,2,4-trimethylquinoline, which has been shown to be effective for stabilizing caro-

tene in alfalfa meal (*7*). The animal fat employed was a white grade, unstabilized tallow containing 2.5% free fatty acid, obtained from a local rendering company.

Preparation of Storage Samples Xanthophyll concentrates and the animal fat were dissolved in hexane to facilitate addition to the mixed feed. To certain samples, aliquots of a solution of the antioxidant in acetone were added. Sufficient additional acetone was added to the mixtures to wet them completely and assure intimate mixing of the xanthophyll and meal. The sample was stirred continuously in a current of air to remove the solvent and assure uniform distribution of the xanthophyll. Animal fat and antioxidant were added to the alfalfa meal in a like manner. Measured amounts of the antioxidant were added directly to portions of the xanthophyll concentrates, which were then warmed briefly on a steam bath and thoroughly mixed.

Xanthophyll concentrates were stored in two ways. One-gram portions were weighed into open-shell vials, which were held in loosely covered baskets in the dark at 25° C. for 4 months. Other portions were stored in sealed 4-ounce bottles, filled to give a minimum amount of head space. Two-gram samples of the alfalfa meal and 4-gram samples of the mixed feeds were weighed into shell vials and stored under the same conditions. The larger samples of mixed feed were required for analysis because of the lower xanthophyll content.

Methods of Assay Xanthophyll and carotene were determined in the alfalfa meal by a procedure developed in this laboratory (*7*). Briefly, the method consists of soaking the sample at room temperature overnight with a mixture of acetone-hexane (3 to 7), chromatographing an aliquot of the extract on a column of magnesium oxide and Celite (1 to 1), and eluting the xanthophyll with alcohol-hexane (1 to 1) after first eluting the carotene with acetone-hexane (1 to 9). The use of this concentration of alcohol, during the chromatography of xanthophyll from an alfalfa extract, has been recently described (*7*).

The xanthophyll concentrates were assayed in a like manner, except that alcohol-hexane (1 to 9) was used to elute the xanthophyll from the chromatographic column. Because of the low xanthophyll content of some of the mixed feed samples, it was necessary to modify the analytical procedure slightly. In these cases the entire extract was filtered through a sintered-glass funnel after the overnight soaking and the meal was washed with small portions of acetone until the filtrate was colorless. Following this, the filtrate was evaporated to dryness under vacuum. The residue was taken up in 10 ml. of acetone-hexane (1 to 9) and a 5-ml. aliquot chromatographed on a column, 12 mm. in inside diameter packed to 70 mm. in height with magnesium oxide and filter aid (1 to 1). The column was developed with acetone-hexane (1 to 9) to elute the carotene and alcohol-hexane (1 to 9) to elute the xanthophyll.

Results of Storage Tests When the xanthophyll concentrates were stored in loosely covered vials, their stability depended on the method of preparation and the source of the concentrate (Table I). The xanthophyll in the saponified concentrate from alfalfa (concentrate A) and the other from corn (concentrate B) were unstable. No carotenoids remained after 2 months' storage at 25° C.

Concentrate C, from an unsaponified alfalfa extract, lost about two thirds of its initial carotene and xanthophyll during the 4-month storage period. The xanthophyll concentrate from lettuce meal (concentrate D) was the most stable of those studied, losing only one third of its xanthophyll and one half of its carotene during the 4-month storage period. When concentrate A was stored in a completely filled, sealed container, there was no appreciable loss of either carotene or xanthophyll after 4 months at 25° C.

The carotenoid stability of concentrate A was improved by addition of the concentrate to broiler feeds 1 and 2 (Table II). At the lower concentrations of 0.5 and 0.25%, the pigment stabilities in the two feeds were about the same. However, at the level of 1%, the added carotenoids were slightly more stable in broiler feed 1.

Earlier studies (*3*) have shown that when increasing amounts of carotene were added to a dry feed carrier, the relative stability of the carotene decreased progressively. A similar effect was found with xanthophyll when increasing amounts of concentrate A were added to a mixed feed (Table II).

The addition of 1% or less of concentrate B from corn gluten meal to broiler feed 1 gave a retention of about one fourth of the xanthophyll and one fifth of the carotene after storage for 4 months (Table III).

The stability of carotenoids in a concentrate from alfalfa prepared without saponification (concentrate C) was about the same after addition to broiler feed 1 as when stored alone (Tables I and III). The carotenoids of the lettuce meal concentrate D were less stable in the mixed feed than when the concentrate was stored alone (Table III).

The use of antioxidant improved the

Table II. Stability of Xanthophyll Concentrate A[a] from Alfalfa Added to Broiler Feeds 1 and 2

Concentrate Added, %	Antioxidant Added, %	Broiler Feed 1: Initial Pigment Concn., P.P.M.		Broiler Feed 1: Retention after 4 Months at 25° C., %		Broiler Feed 2: Initial Pigment Concn., P.P.M.		Broiler Feed 2: Retention after 4 Months at 25° C., %	
		Xanthophyll	Carotene	Xanthophyll	Carotene	Xanthophyll	Carotene	Xanthophyll	Carotene
10	0	317	222	0	0	..	..	..	..
	0.015	317	244	2	1	..	..	..	..
	0.125	311	271	37	21	..	..	..	..
5	0	153	113	0.6	0.3	..	..	..	..
	0.015	175	140	11	4	..	..	..	..
	0.125	186	143	79	67	..	..	..	..
1	0	52	35	16	6	26	20	3	2
	0.015	51	37	58	41	30	23	74	80
	0.125	50	34	83	93	27	23	79	97
0.5	0	36	25	14	5	16	11	10	8
	0.015	35	26	69	53	17	13	81	93
	0.125	37	29	90	84	17	13	89	100
0.25	0	31	21	13	6	9	8	8	14
	0.015	32	19	70	67	9	8	94	79
	0.125	31	24	89	83	9	9	100	99

[a] Saponified.

Table III. Stability of Various Xanthophyll Concentrates Added to Broiler Feed[a]

Concentrate Added, %	Antioxidant Added, %	Animal Fat Added, %	Concentrate B from Corn Gluten Meal: Initial Pigment Concn., P.P.M. Xanthophyll	Concentrate B from Corn Gluten Meal: Initial Pigment Concn., P.P.M. Carotene	Concentrate B from Corn Gluten Meal: Retention after 4 Months at 25° C., % Xanthophyll	Concentrate B from Corn Gluten Meal: Retention after 4 Months at 25° C., % Carotene	Concentrate C[b] from Alfalfa Meal: Initial Pigment Concn., P.P.M. Xanthophyll	Concentrate C[b] from Alfalfa Meal: Initial Pigment Concn., P.P.M. Carotene	Concentrate C[b] from Alfalfa Meal: Retention after 4 Months at 25° C., % Xanthophyll	Concentrate C[b] from Alfalfa Meal: Retention after 4 Months at 25° C., % Carotene	Concentrate D[b] from Lettuce Meal: Initial Pigment Concn., P.P.M. Xanthophyll	Concentrate D[b] from Lettuce Meal: Initial Pigment Concn., P.P.M. Carotene	Concentrate D[b] from Lettuce Meal: Retention after 4 Months at 25° C., % Xanthophyll	Concentrate D[b] from Lettuce Meal: Retention after 4 Months at 25° C., % Carotene
1	0	0	50	17	25	19	208	37	32	24	55	27	37	24
	0.015	0	52	18	62	64	207	38	61	62	64	31	62	60
	0.125	0	52	18	90	97	209	37	86	100	65	33	79	94
1	0	5	47	18	52	41	206	35	51	54	61	31	56	50
	0.125	5	47	19	98	94	207	37	93	100	61	32	86	100
0.25	0	0	30	13	28	19	62	18	32	27	32	17	31	20
	0.015	0	32	13	66	67	66	19	72	75	36	19	67	66
	0.125	0	34	14	83	98	71	20	79	96	36	20	82	94

[a] Broiler feed 1.
[b] Unsaponified.

Table IV. Stability of Xanthophyll in Alfalfa Meal

Antioxidant Added, %	Animal Fat Added, %	Initial Pigment Concn., P.P.M. Xanthophyll	Initial Pigment Concn., P.P.M. Carotene	Retention after 4 Months at 25° C., % Xanthophyll	Retention after 4 Months at 25° C., % Carotene
0	0	548	272	64	37
	1	523	265	70	45
	5	503	255	74	62
0.015	0	543	271	79	59
0.125	0	564	270	80	72
0.015	1	537	275	79	62
0.125	1	511	269	86	72
0.015	5	521	261	79	80
0.125	5	503	258	85	91

stability of the pigments, giving a several-fold increase in retention in all cases where the stabilizer was used.

The addition of 5% of animal fat to the broiler feeds resulted in a doubling of the retention of both carotene and xanthophyll (Table III). Even more effective was the addition of both antioxidant and animal fat, resulting in almost complete retention of the added carotenoids.

In the alfalfa meal, both the xanthophyll and carotene were more stable than in any of the mixed feeds (Table IV). The addition of antioxidant increased the stability but the degree of increase was not so pronounced as in the mixed feeds because of the greater initial stability in the untreated meal.

Discussion As previously shown (*3*), several factors may influence the relative stability of a xanthophyll concentrate when added to a mixed feed. These include the possible presence of natural antioxidants in the feed as well as in the concentrate, the initial carotenoid content, and the severity of conditions of exposure to oxygen that occur in a finely divided material such as a mixed feed. The final stability of the carotenoids will depend on the relative importance of these factors as well as the inherent stability of the concentrate itself.

It is evident that there are considerable differences in the relative stabilities of commercial xanthophyll concentrates. Apparently, these differences in stability may be caused by the method of preparation or because the source material initially had little or no natural antioxidants. During the commercial preparation of concentrates, treatments such as saponification may remove or destroy most of the naturally present antioxidants such as tocopherols.

Probably it was the presence of natural antioxidants in certain components of the mixed feed such as dehydrated alfalfa meal that resulted in an improvement in the stability of the added carotenoids. Since larger amounts of natural antioxidants are already present in the more stable concentrates, the antioxidants present in the feed exert relatively less effect than they do with the less stable concentrate.

Because the conditions of exposure to oxidation in the mixed feed were too severe for the natural antioxidants to give a complete stabilizing effect, a more potent antioxidant such as 6-ethoxy-1,2-dihydro-2,2,4-trimethylquinoline was able to give enhanced carotenoid stability in the mixed feeds. The addition of an animal fat was likewise effective in promoting greater carotenoid stability in the mixed feeds, probably by bringing the natural antioxidants and carotenoids into more intimate contact.

Summary

In the absence of added antioxidant, the pigments of xanthophyll concentrates in mixed feeds were unstable. The stability of the carotenoids varied depending on source and was reduced by saponification of the concentrates during preparation.

Both animal fats and an antioxidant, 6 - ethoxy - 1,2 - dihydro - 2,2,4 - trimethylquinoline, acted as effective stabilizers for the added xanthophylls in mixed feeds. The two used together were more effective than either one used separately.

Acknowledgment

The technical assistance of I. V. Ford is gratefully acknowledged. The authors are indebted to the Poultry Producers of Central California, Petaluma, Calif., and Albers Milling Co., Oakland, Calif., for broiler mash and dehydrated alfalfa meal.

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Received for review December 20, 1954. Accepted February 7, 1955.