Ethoxyquin and Egg Yolk Pigmentation. (28340)

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Although birds resemble mammals in their ability to convert carotene to Vitamin A, xanthophylls are stored in the liver, body fat, skin, feathers, shanks, etc., in contrast to the deposition of beta-carotene in mammals (1,2). The xanthophylls are also deposited in egg yolks to a greater extent than carotene(3). As early as 1919, Palmer and Kempster(4) reported that yolk pigmentation depended upon the carotenoids in the diet of the laying hen.

Potter and Bunnell(5) found that the antioxidant N,N'-Diphenyl-p-phenylenediamine (DPPD) fed with dehydrated alfalfa meal increased the concentrations of Vit. A and carotenoids in the plasma and livers of chicks. The effect of the antioxidant appeared to be associated with the stabilization of the carotenoid pigments in the diet and possibly *in vivo*. High *et al.*(6) suggested that the site of action of antioxidants was in the intestinal wall or at the site of conversion of carotene to Vit. A. The feeding of antioxidants has been shown to reduce the dietary requirements for Vit. A(7). Siedler *et al.*(8) indicated the reduced Vit. A requirement was due to decreased destruction of Vit. A and carotene in the diet. The antioxidants DPPD and Butylated Hydroxytoluene (BHT), when compared as aids in the deposition of carotenoid pigments in the skin of broilers, appeared to be equal in effectiveness(7). Ethoxyquin was reported by Gordon and Machlin(9) to be superior to BHT in preservation of Vit. A for growing chickens.

The present studies were undertaken to evaluate the degree of xanthophyll stabilization in feed and to determine the effect of the antioxidant, ethoxyquin, on egg yolk pigmentation.

Methods. Single Comb White Leghorn Pullets, housed in individual cages, were placed on a low xanthophyll diet (Table I) for a 4-week pre-experimental period prior to initiation of the respective experiments. At the end of this 4-week period, the egg yolks had decreased in pigments to a minimum value and the respective dietary treatments were imposed. All diets were isonitrogenous at 16.5% crude protein and isocaloric at 869 Calories of productive energy per pound.

TABLE I. Low Pigment Basal Diet (Diet 1).

Ingredient	%
White corn, ground	56.5
Soybean meal (44% protein; solvent)	21.0
Dried whey (12% protein)	2.0
Dicalcium phosphate*	2.5
Vitamin premix†	2.5
Ground limestone	5.0
Sodium chloride	.5
Manganese sulfate pentahydrate (70%)	.02
Test ingredient or wheat bran	10.00
Total	100.02

* Dynafos, International Minerals & Chemical Co., Skokie, Ill.

† Supplied the following per lb of diet: 4,500 I.U. vit A, 700 I.C.U. vit D_3, 2.0 mg riboflavin, 12.5 mg niacin, 5.0 mg D-calcium pantothenate, 200 mg choline chloride, 6 μg vit B_{12}, 2.5 I.U. alpha-tocopheryl acetate, 1.0 mg menadione sodium bisulfite, 2.0 mg procaine penicillin, and 10.0 mg chlortetracycline.

In the first experiment, dehydrated alfalfa meal was fed at levels up to 6.25% in the presence and absence of 125 ppm ethoxyquin for a period of 3 weeks. All eggs laid were collected daily and analyzed for acetone extractable pigments in the yolk according to the method outlined by A.O.A.C.(10). The acetone extracts of the pigments were read in an Evelyn colorimeter at 440 mμ and calculated as beta-carotene. A second experiment conducted during the colder portion of the year essentially duplicated the procedures involved in the first study in order to evaluate the effect of ambient temperature on the results obtained with the antioxidant feeding.

The third experiment was designed to determine the effectiveness of different modes of ethoxyquin administration on the result-

TABLE II. Effect of Ethoxyquin on Concentration of Carotenoid Pigments in Egg Yolks from Hens Fed Dehydrated Alfalfa Meal as the Sole Source of Carotenoid Pigments (July-Aug).

Dietary dehydrated alfalfa meal (%)	Concentration of acetone extractable pigments (μg/g of yolk)* Without ethoxyquin	With ethoxyquin (0.0125%)	% increase with ethoxyquin
.00	.85[a]	.90[ab]	—
1.25	1.87[b]	3.24[c]	73.26
2.50	3.71[c]	5.26[d]	42.00
3.75	5.83[d]	9.11[f]	60.96
5.00	6.86[e]	11.01[g]	60.49
6.25	9.40[f]	12.42[h]	32.13
Avg	4.75	6.99	53.76

* Means having different superscripts are significantly different at the .01 level of probability.

ing xanthophyll in egg yolks. To evaluate the possible site of xanthophyll destruction *in vivo*, ethoxyquin was administered in 3 ways: (1) along with experimental diet at the rate of 125 ppm; (2) orally, 3 times daily, by capsule, at 50 mg per dose; (3) intramuscularly, 3 times daily, at 50 mg per injection. The experimental diets were mixed to contain 5, 10, and 20% dehydrated alfalfa meal as a source of xanthophylls and maintained isocaloric and isonitrogenous by adjusting soybean meal, white corn and glucose monohydrate.

Results and discussion. Experiment 1. The maximum degree of xanthophyll concentration in the egg yolks of hens fed dehydrated alfalfa meal was obtained after 12 days on the respective diets. At this time the degree of yolk pigmentation essentially reached a plateau. The values from 10 consecutive days into plateau region were employed to evaluate the effectiveness of the dietary treatments.

In the absence of dehydrated alfalfa meal in the diet, the acetone extractable pigment concentration per gram of egg yolk was 0.85-0.90 μg. A statistically significant increase in yolk pigment concentration was obtained with the feeding of each increasing increment of dehydrated alfalfa meal in the absence of ethoxyquin (Table II). The feeding of 125 ppm ethoxyquin in addition to dehydrated alfalfa meal resulted in a statistically higher level of yolk pigmentation at each level of dehydrated alfalfa meal fed. The average improvement due to the feeding of ethoxyquin was 53.8%.

Beta-carotene and xanthophyll analyses on the respective experimental diets, initially and at termination of the 3-week experimental period, indicated an average loss of 32% in beta-carotene and 24% in the xanthophyll components when ethoxyquin was not included in the diet (Table III). It would, therefore appear that a portion of the improved yolk pigmentation obtained as a result of ethoxyquin feeding could be attributed to the preservation of dietary xantho-

TABLE III. Retention of Beta-Carotone and Xanthophylls in Mixed Feed After 3 Weeks Storage in Presence of Ethoxyquin* (July-Aug).

Dietary dehydrated alfalfa meal (%)	Without ethoxyquin: Beta-carotene (mg/lb)	Beta-carotene (% loss)	Xanthophylls (mg/lb)	Xanthophylls (% loss)	With ethoxyquin†: Beta-carotene (mg/lb)	Xanthophylls (mg/lb)
1.25	.69	56.87	2.53	—	1.60	2.55
2.50	1.12	18.12	3.72	33.33	1.38	5.58
3.75	1.46	37.33	5.40	20.58	2.33	6.80
5.00	1.83	—	6.80	—	—	—
6.25	2.66	16.35	7.68	20.17	3.18	9.62
Avg		32.16		24.01	2.12	6.14

	Temp‡ (°F)	Relative humidity‡ (%)
Mean max (July-Aug)	102.0–96.0	64.9–76.3
" min " "	77.3–74.5	25.9–33.5

* Initial values for beta-carotene and total xanthophylls in the mixed feed were esentially the same as reported for the ethoxyquin containing diets.

† 0.0125% ethoxyquin in the feed.

‡ Recorded on a hygrothermograph. The Instrument Corp., Baltimore, Md.

TABLE IV. Effect of Ethoxyquin on Yolk Pigmentation and Retention of Beta-carotene and Xanthophylls in Mixed Feed After 2 Weeks Storage (Nov-Dec).

	Dietary dehydrated alfalfa meal (%)					
	1.25		3.75		6.25	
Antioxidant*	With	Without	With	Without	With	Without
Acetone extractable pigments (μg/g yolk) †	2.10[a]‡	1.70[a]	4.46[c]	3.64[b]	4.84[d]	4.06[c]
Yolk pigmentation, % increase with ethoxyquin	23.5		22.5		19.2	
Initial beta-carotene (mg/lb)	.21	.21	.63	.63	.69	.69
Initial xanthophylls (mg/lb)	2.15	2.15	4.60	4.60	4.87	4.87
Final dietary beta-carotene (mg/lb)	.27	.13	.63	.49	.66	.59
Final dietary xanthophylls (mg/lb)	1.89	2.06	4.69	4.65	4.93	4.81
	Temp (°F)		Relative humidity (%)			
Mean max (Nov-Dec)	73.9–68.5		78.4–82.8			
" min " "	47.9–41.4		36.3–37.2			

* Ethoxyquin at 0.0125% of diet.
† Calculated as beta-carotene.
‡ Means having different superscripts were significantly different at the .01 level of probability.

phylls. However, an *in vivo* effect of the antioxidant was also indicated.

The temperature extremes existing during this study were maximum range 96-102°F and minimum range 74.5-77.3°F.

Experiment 2. The results of the second experiment, conducted during periods of 68.5-73.5°F maximum temperature and 41.4-47.9°F minimum temperature, again indicated a significant improvement in yolk pigmentation as a result of feeding laying hens a diet containing 125 ppm ethoxyquin and dehydrated alfalfa meal (Table IV). The average percentage improvement in yolk pigmentation amounted to 19.2-23.5% when ethoxyquin was fed. There was essentially no loss in dietary carotene or xanthophylls throughout the experimental period as evidenced by feed analyses data (Table IV). These data indicate that the *in vivo* loss of xanthophylls in each of the 2 experiments conducted was approximately 20%.

Experiment 3. Statistically significant improvements in yolk pigments were obtained through the feeding of 5, 10 and 20% dehydrated alfalfa meal in the presence of ethoxyquin. The highest degree of yolk pigmentation was obtained with the 20% dehyd. alfalfa meal diet containing the antioxidant (Table V). The feeding of 20% dehydrated alfalfa meal in the absence of ethoxyquin resulted in only 80% of the response obtained with ethoxyquin in the diet. A significant increase in xanthophyll deposition was obtained by administration of the antioxidant either parenterally or by capsule, as compared with

TABLE V. Effect of Mode of Ethoxyquin Administration on Egg Yolk Pigmentation.

Treatment		Initial mg xanthophylls/lb of diet†	μg of acetone extractable pigment/g of egg yolk
Per cent dehy. alfalfa meal	Antioxidant		
5 0	+*	7.03	10.1[a]‡
10.0	+	13.7	19.3[b]
20 0	+	25.9	35.7[c]
20.0	Capsule 3 × daily§	25.9	31.0[d] (89%)‖
20.0	Injected 3 × daily§	25.9	31.3[d] (89%)
20.0	—	25.9	28.2[e] (79%)

* Ethoxyquin at .0125% of diet.
† Calculated as beta-carotene.
‡ Means having different superscripts are significant at .05 level of probability.
§ Ethoxyquin—50 mg/dose.
‖ Percentage of 20% alfalfa level with dietary antioxidant.

feeding of the 20% dehydrated alfalfa meal in the absence of the antioxidant. The responses obtained with these 2 ethoxyquin treatments amounted to 89% of that obtained with the antioxidant in the diet.

The loss of dietary xanthophylls during the experimental period amounted to 12% in the absence of ethoxyquin. These data suggest that the oxidative destruction of xanthophylls in the digestive tract of the laying hen was negligible since neither oral nor parenteral administration of ethoxyquin resulted in identical levels of yolk pigmentation. However, the possibility exists that the injected antioxidant was excreted into the gut and thereby served to protect the xanthophylls.

Summary. Three experiments have been conducted to evaluate the site of action and effectiveness of the antioxidant ethoxyquin on egg yolk pigmentation. Results of the first experiment conducted during a period of high ambient temperatures indicated an average improvement of 53.8% in yolk pigmentation due to the feeding of 125 ppm of the antioxidant in addition to dehydrated alfalfa meal in the diet of laying hens. In the absence of ethoxyquin, dietary loss of xanthophylls amounted to 24% and loss of beta-carotene was 32%.

The second experiment conducted during cooler weather resulted in an improvement of 21% in yolk pigmentation due to ethoxyquin feeding. Dietary losses of carotenoids in the second study were negligible.

The result of the third experiment indicated approximately 10% of the xanthophylls were destroyed *in vivo*. This *in vivo* loss of xanthophyll pigments was prevented by either the feeding of 125 ppm ethoxyquin in the diet or administration of the antioxidant by capsule (50 mg, 3 times daily) or intramuscular injection (50 mg, 3 times daily).

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β_{2A} and Other Immunoglobulins in Isolated Anti-A Antibodies.* (28341)

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A considerable body of evidence indicates that the β_{2A} globulins probably represent a third class of antibody proteins in addition to the well-established 7S and 19S γ-globulins(1,2). However, the clear delineation of antibody activity in this serum protein fraction has proven very difficult primarily because of the problems involved in isolating β_{2A} globulins which are completely free of

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