Utilization of Xanthophylls from Natural Sources by the Chick

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Studies were conducted with chicks to compare the relative utilization of xanthophylls from dehydrated alfalfa meal, corn gluten meal, commercial yellow corn, and new strains of high-xanthophyll corns. Both commercial and a new strain of high-xanthophyll corn were equally effective, but they produced greater pigmentation than alfalfa meal and corn gluten meal when each of these ingredients was used to furnish comparable dietary xanthophyll levels. No significant differences among dietary treatments were shown in the absorption of total xanthophylls when the output of these pigments in the feces was measured. Increasing the dietary xanthophyll levels resulted in a linear increase in the skin and serum xanthophylls; per cent absorption of xanthophylls was negatively correlated with the dietary xanthophyll levels. Strains of corn having different amounts of main individual pigments, lutein and zeaxanthin, were essentially similar in their pigmenting effect.

Xanthophylls, the hydroxycarotenoids, are important constituents of certain foodstuffs, as they impart desirable yellow color to skin and egg yolk of poultry. Dehydrated alfalfa meal, yellow corn (*Zea mays* L.), and corn gluten meal are the major natural sources of these pigments used in present poultry diets. Xanthophylls from yellow corn are reported to be better utilized by the chick for skin deposition of pigments than those from dehydrated alfalfa meal and corn gluten meal (*5, 11*). The reasons for the differences in the biological availability of these pigments from different sources have not been explained. Different extraction methods used for the determination of xanthophylls, chemical assay errors, variation in the individual types of xanthophylls, and differences in their absorption from the intestinal tract are some possible reasons why the pigments from some feedstuffs are more effective biologically than others.

New strains of yellow corn high in xanthophylls are being developed under a corn breeding program. Some of these strains have two to three times the xanthophylls present in commercial yellow corn. In a previous study (*10*), xanthophylls from new varieties and from commercial yellow corn were equally available to the chick for skin pigmentation when used to supply comparable levels of dietary xanthophylls.

The present studies were conducted to compare further the relative utilization of xanthophylls from dehydrated alfalfa meal, corn gluten meal, commercial yellow corn, and a new strain of high-xanthophyll yellow corn. Balance studies were conducted to determine if the differences in the availability of xanthophylls are related to the differences in the absorption of these pigments from the intestinal tract. A study also was conducted to compare new strains of yellow corn containing different levels of individual pigments, lutein and zeaxanthin.

Procedure

Three experiments were conducted with broiler-type male chicks (Vantress X Arbor Acre) which were housed as groups in battery brooders with raised wire-screen floors. Day-old chicks were fed a carotenoid-free diet (a white corn–soybean meal type starting diet) for 1 week (experiments 1 and 3) or 2 weeks (experiment 2) before they were given experimental diets. Each experimental diet was fed to duplicate groups of 10 chicks each in experiments 1 and 2, and 5 chicks each in experiment 3. The diets and water were given *ad libitum*. Individual body weights were obtained at the beginning and end of the test periods. Feed consumption records were obtained by groups.

Composition of the basal diet used in experiment 1 is shown in Table I (diet A). Chromic oxide was included as an inert index substance to facilitate the determination of carotenoid absorption (*6*). Dehydrated alfalfa meal (20% protein), corn gluten meal (40% protein), commercial yellow corn, and a new strain of high-xanthophyll yellow corn designated as experimental yellow corn were compared in this experiment. These ingredients were analyzed for their total xanthophyll content before use. Alfalfa meal, corn gluten meal, commercial yellow corn, and the experimental yellow corn contained by chemical analysis 366, 66, 24, and 40 mg. of xanthophylls per kg., respectively. On the basis of their analysis, each of these ingredients was substituted singly in the basal diet, at levels to supply 6.6 and 11.0 mg. of xanthophylls per kg. of diet. The substitutions in the basal ration were made in such a way that alfalfa meal was substituted for wheat shorts, corn gluten meal for soybean meal, and commercial and experimental yellow

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Table I. Composition of Basal Diets

Ingredient	Diet A, %	Diet B, %
Ground white corn	56.04	52.20
Soybean meal, 50% protein	30.00	27.60
Fish meal, 60% protein	2.50	2.50
Poultry by-products meal, 55% protein	2.50	2.50
Wheat middlings	3.05	...
Soybean mill feed	...	9.50
Vegetable oil[a]	3.00	3.00
Defluorinated rock phosphate, 14% P, 34% Ca	1.50	1.50
Limestone	0.50	0.25
Sodium chloride	0.30	0.30
Premix	0.31[b]	0.35[c]
Chromic oxide	0.30	0.30
	100.00	100.00

[a] Wesson oil, Wesson Oil Sales Co., Fullerton, Calif.
[b] Supplied following per kg. of diet: vitamin A, 3300 international units; vitamin D_3, 1200 international chick units; riboflavin, 4.4 mg.; Ca pantothenate, 8.8 mg.; niacin, 35.2 mg.; vitamin B_{12}, 9.9 μg; α-tocopheryl acetate, 13.2 international units; manganese sulfate, 141.0 mg.; menadione sodium bisulfite complex, 2.2 mg.; ethoxyquin, 99.9 mg.; DL-methionine, 250.9 mg.; choline chloride, 325.0 mg.
[c] Supplied same micronutrients as for diet A except that DL-methionine was increased to 650.4 mg./kg. of diet.

corns for white corn. The experimental diets were fed from 1 to 8 weeks of age. From 5 to 8 weeks of age, the chicks were kept in unheated batteries with raised wire-screen floors, and the protein level of each experimental diet during this period was reduced approximately by 2.5% by changing the levels of white corn and soybean meal.

During the last week of the experiment, the feces which fell onto pans covered with waxed paper were collected for three consecutive days. Each day's fecal collections were stored in a refrigerator. Fecal collections for 3 days were composited and portions frozen for future analysis.

A second experiment was conducted to study the effect of increasing dietary levels of xanthophylls on their utilization and absorption and to compare further the relative availability of xanthophylls from dehydrated alfalfa meal and corn gluten meal. The corn gluten meal (60% protein) used in this experiment had a much higher content of xanthophylls than the corn gluten meal used in the first experiment. According to chemical assays, alfalfa meal and corn gluten meal contained 282 and 264 mg. of xanthophylls per kg., respectively. Graded levels of both dehydrated alfalfa and corn gluten meal were used to supply, singly, 11, 22, 33, and 44 mg. of xanthophylls per kg. of diet. The basal diet (Table I, diet B) was similar to the one used in the first experiment, except that wheat shorts were removed and soybean mill feed was used in the diets containing corn gluten meal to equalize the fiber level of all diets. These diets also were formulated to contain almost similar levels of the amino acids, methionine, and lysine. The test diets were fed for 3 weeks. Feces for examination were collected for 3 consecutive days during the last week of the experiment.

A third experiment was conducted to compare the availability of the pigments from high-xanthophyll strains of yellow corn which were developed to have different levels of the principal individual pigments, lutein and zeaxanthin. The analyses of those strains used in the feeding experiment are shown in Table II. On the basis of their analysis, they were designated as high zeaxanthin–low lutein, equal zeaxanthin and lutein, and low zeaxanthin–high lutein. Since only small amounts of each strain were available, strains within each sample designation had to be pooled. On the basis of their total xanthophyll values, the three corn samples were included in the basal diet to supply separately 5.5 mg. of total xanthophylls per kg. of diet. The basal diet was similar to the one used in experiment 1, in which these strains of yellow corn replaced an equal amount of white corn. Since no balance studies were planned in this experiment, chromic oxide was omitted from these diets and the same amount of white corn was added to make the diets 100%. The experimental diets were fed to week-old chicks up to 5 weeks of age.

At the termination of the test periods, the birds were bled by cardiac puncture, then killed by disjunction of the neck, and their feet were removed. Serum and skin samples from each of five birds were pooled for determination of xanthophylls.

The experimental diets were stored in a cooler to minimize any loss of pigments during the test periods. The carotenoid analysis of diets at the start and end of the experimental periods did not show any loss of pigments during this period. The carotenoid values obtained by actual analysis at the start were used for calculating per cent absorption of carotenoids.

The analytical procedure for determining carotenoids of yellow corn and corn gluten meal was essentially that of Blessin (*3*), except that a Goldfish fat extraction apparatus rather than Butt extraction tubes was used to extract the pigments. Diet samples containing these ingredients also were analyzed by the same procedure. Dehydrated alfalfa meal and the diet samples containing this ingredient were analyzed by the method of Bickoff *et al.* (*2*). Feces samples were saponified with alcoholic KOH and carotenoids were then extracted with hexane.

Table II. Carotenoid Content of High Xanthophyll Strains of Yellow Corn

(Micrograms per gram)

Sample Designation	Carotene	Xanthophylls	Lutein	Zeaxanthin
High zeaxanthin–low lutein	4.0	47.5	9.1	29.8
	6.9	60.0	15.6	31.0
	5.4	53.2	12.3	33.0
Equal zeaxanthin-lutein	7.6	55.6	22.1	20.0
	6.2	55.2	25.2	21.2
Low zeaxanthin–high lutein	6.1	49.6	27.2	13.5
	5.2	45.1	24.0	11.5

The hexane extract was reduced in volume under pressure and applied on a chromatographic column for the separation of main fractions, carotenes and xanthophylls (*2*). The method of Quackenbush *et al.* (*9*) was employed to estimate the individual pigments, lutein and zeaxanthin, in the high-xanthophyll strains of corn. Skin xanthophylls were determined as described by Day and Williams (*5*). Serum xanthophylls were determined by the method of Grau and Klein (*8*). Chromic oxide was determined in feed and feces samples according to the method of Czarnocki, Sibbald, and Evans (*4*).

Statistical examinations of the data were made by analysis of variance according to Steel and Torrie (*12*) and treatment differences determined by Duncan's multiple range test (*7*). Part of the data from the second experiment also was evaluated by regression analysis techniques.

Results and Discussion

The results of experiment 1 are shown in Table III. Body weight and feed utilization were depressed in the groups fed the diet containing the higher level of corn gluten meal. This level of corn gluten meal, used to furnish the higher xanthophyll level, probably resulted in certain amino acid deficiencies. In all other groups, body weight and feed efficiency were essentially similar.

Within each dietary xanthophyll level, skin and serum xanthophylls were greater in the groups in which diets containing yellow corn were used as compared with those fed dehydrated alfalfa meal and corn gluten meal. The over-all means showed no significant differences in serum and skin xanthophylls between the two groups fed yellow corn, but they were significantly ($P < 0.05$) greater than the groups furnishing xanthophylls from alfalfa meal and corn gluten meal. Although skin and serum xanthophylls were higher for the birds fed corn gluten meal than for those fed alfalfa meal, they were not statistically different. No difference in the absorption of xanthophylls was found among different treatments at either dietary level of xanthophylls.

The results agree with previous reports which indicate that the xanthophylls furnished by yellow corn are more efficiently utilized than those of alfalfa meal and corn gluten meal. These results also agree with those of Ratcliff *et al.* (*10*), that the xanthophylls from commercial yellow corn and a new strain of high-xanthophyll yellow corn were equally available. Although there was no significant difference between the groups fed the two yellow corns, about 40% less of the high-xanthophyll corn was used to supply the same xanthophyll level as the commercial yellow corn. The high-xanthophyll corn could be used to furnish the sole source of dietary xanthophylls of a broiler ration containing the standard 60% corn.

The difference in the biological availability of xanthophylls from these sources could not be explained on the basis of differences in the absorption of total xanthophylls from the intestinal tract as measured in this study. The xanthophylls present in these ingredients are very complex in nature. For example, Bickoff *et al.* (*1*) reported the presence of more than 40 xanthophyll bands in dehydrated alfalfa meal. The five main pigments—lutein, violaxanthin, cryptoxanthin, zeaxanthin, and neoxanthin—comprised 87% of the xanthophylls of dehydrated alfalfa meal. Similarly, wide differences of individual pigments exist in the yellow corn. The differences in the individual types of xanthophylls, differences in the rate of absorption, and their interactions are some possible explanations for the failure to find a difference in the absorption of the total xanthophylls. Obviously, more work is needed to understand the basic metabolism of the individual pigments present in these sources. The possibility exists also that there may be greater oxidation or destruction of xanthophylls in the gastrointestinal tract from alfalfa meal, as compared to those present in yellow corn. The per cent absorption as measured in this study takes into account the intake and output of total xanthophylls. It also includes any destruction of xanthophylls in the intestinal tract. A significant ($P < 0.01$) correlation coefficient, $r = 0.874$,

Table III. Effect of Dietary Modifications on Utilization of Xanthophylls by the Chick
(1 to 8 weeks, experiment 1)

Source and Dietary Level of Xanthophylls	Body Wt. Gain, G.	Feed Gain	Skin Xanthophylls[a] μg./100 Sq. Cm.	Serum Xanthophylls[a] μg./Ml.	Xanthophyll[b] Absorption, %
6.6 mg./kg.					
Alfalfa meal (1.8)[c]	1300	2.19	63	1.9	54
Corn gluten meal (10.0)	1315	2.25	71	2.2	52
Commercial yellow corn (27.5)	1340	2.18	98	3.8	53
Experimental yellow corn (16.5)	1310	2.18	94	3.6	54
11.0 mg./kg.					
Alfalfa meal (3.0)	1351	2.35	95	3.9	56
Corn gluten meal (16.7)	1046	2.64	105	4.0	49
Commercial yellow corn (45.8)	1332	2.26	137	6.0	51
Experimental yellow corn (27.5)	1327	2.18	126	5.8	53

[a] Each value represents average of four separate determinations (samples from five birds pooled for each determination).
[b] Calculated using chromic oxide ratio technique during last week of experiment.
[c] Per cent of diet.

was calculated between skin and serum xanthophylls in this experiment.

The results of the second experiment, in which increasing dietary levels of xanthophylls furnished by alfalfa meal and corn gluten meal were used, are shown in Table IV. Both body weight and feed efficiency were better for the groups fed corn gluten meal as compared to those fed alfalfa meal. Skin and serum xanthophylls were increased with each increase in dietary xanthophyll level. There was little difference in the skin and serum xanthophyll values between the two groups fed the two higher levels of xanthophylls furnished by alfalfa meal. In general, the skin and serum xanthophylls were higher for the birds fed corn gluten meal than for those fed alfalfa meal. The analysis of variance, however, did not show any significant difference in skin and serum xanthophylls owing to the source of dietary pigments.

Xanthophyll absorption tended to be depressed with increasing dietary xanthophyll levels. The decreased absorption with increasing dietary levels of xanthophylls could be due to overloading the absorption sites in the intestinal tract. This explains the lowered efficiency of xanthophyll utilization with the increase of dietary xanthophyll levels (*10*, *11*).

The effect of increasing dietary levels of xanthophylls on the skin and serum xanthophylls, and the per cent absorption of xanthophylls, were evaluated also by using the regression analysis techniques (Figures 1, 2, and 3, respectively). Since there was no significant difference between the two sources of xanthophylls, common prediction equations were calculated. Individual observations were used in the calculations of the prediction equations, and the average values obtained with alfalfa meal and corn gluten meal were then plotted. Skin and serum xanthophylls were positively correlated ($P < 0.01$) with dietary xanthophyll level ($r = 0.925$ and 0.841, respectively); xanthophyll absorption was negatively correlated ($P < 0.01$) with increasing dietary xanthophyll levels ($r = -0.742$). A significant ($P < 0.01$) correlation coefficient, $r = 0.922$, was calculated between skin and serum xanthophylls.

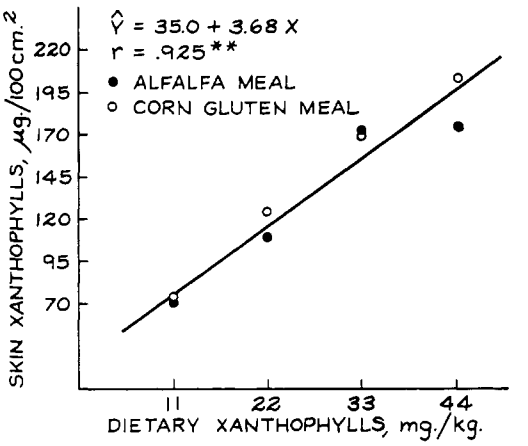

Figure 1. Relationship between dietary level of xanthophylls and skin xanthophylls

Experiment 2. ** = $P < 0.01$

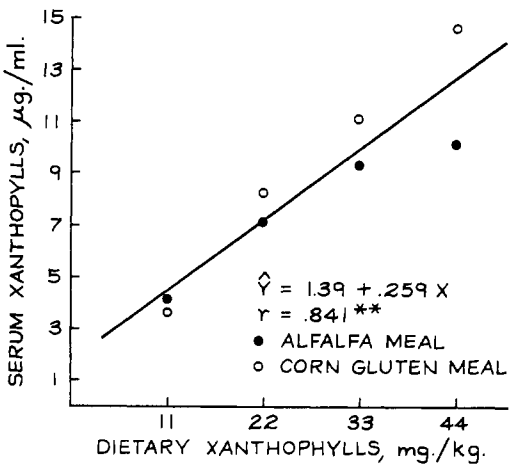

Figure 2. Relationship between dietary level of xanthophylls and serum xanthophylls

Experiment 2. ** = $P < 0.01$

Table IV. Effect of Source and Increasing Dietary Levels of Xanthophylls on Their Utilization by Chick
(2 to 5 weeks, experiment 2)

Source of Xanthophylls	Dietary Xanthophyll Level, Mg./Kg.	Body Wt. Gain, G.	Feed Gain	Skin Xanthophylls[a] µg./100 Sq. Cm.	Serum Xanthophylls[a] µg./Ml.	Xanthophyll[b] Absorption, %
Alfalfa meal						
3.9[c]	11	578	2.11	70	4.1	49
7.8	22	559	2.15	110	7.0	39
11.7	33	552	2.16	172	9.6	46
15.6	44	552	2.18	173	10.0	35
Corn gluten meal						
4.2	11	598	2.04	71	3.6	47
8.3	22	574	2.08	125	8.1	46
12.5	33	591	2.02	167	11.0	41
16.7	44	576	1.98	202	14.9	33

[a] See footnote [a], Table II. [b] See footnote [b], Table II. [c] Per cent of diet.

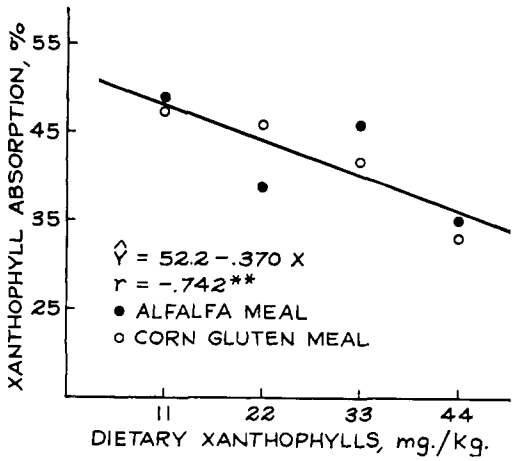

Figure 3. Relationship between dietary level of xanthophylls and xanthophyll absorption

Experiment 2. ** = $P < 0.01$

Table V. Relative Absorption of Carotene and Xanthophyll in Chicks Fed Graded Levels of Dehydrated Alfalfa Meal

(Experiment 2)

Dietary Level of Alfalfa Meal, %	Dietary Level of Carotenoids, Mg./Kg. of Diet		Absorption, %[a]	
	Carotene	Xanthophylls	Carotene	Xanthophylls
3.9	5.5	11.0	72	49
7.8	11.0	22.0	62	39
11.7	16.5	33.0	67	46
15.6	22.0	44.0	49	35

[a] Carotene absorption significantly greater than that of xanthophyll ($P < 0.01$).

Table VI. Utilization of Xanthophylls by Chicken from Different Strains of Yellow Corn

(1 to 5 weeks, experiment 3)

Xanthophyll Source	Skin Xanthophylls[a], μg./G.	Serum Xanthophylls, μg./Ml.
High zeaxanthin–low lutein	5.3	3.6
Equal zeaxanthin–lutein	4.5	2.9
Low zeaxanthin–high lutein	5.9	4.3

[a] Skin from shank area.

Since carotene has to be separated from the xanthophylls in the analysis for these pigments, the carotene content of feed and feces was recorded, and carotene absorption was calculated. This comparison was possible only in groups fed alfalfa meal. Since the carotene content of corn gluten meal is much lower than that of dehydrated alfalfa meal (and with the quantity of samples taken for fecal analysis), the carotene content of the feces in the groups fed corn gluten meal could not be estimated accurately. Table V compares absorption of carotenes and xanthophylls. Per cent absorption of carotene was significantly ($P < 0.01$) greater than that of xanthophyll. Carotene absorption tended to be depressed with increasing dietary levels of carotene.

The results obtained with the feeding of different strains of yellow corn containing different levels of the main pigments, lutein and zeaxanthin, are shown in Table VI. The three sources of yellow corn appeared equally effective for skin and serum xanthophylls. The method of determining skin xanthophylls was slightly modified in this experiment. Portions of the shank skin were removed and weighed; pigments were then extracted with acetone and determined spectrophotometrically. The method, however, correlated very well with the serum xanthophylls. The results from the third experiment cannot be considered entirely conclusive because of the small numbers involved. The development of strains having even greater differences in the principal individual pigments, lutein and zeaxanthin, may show clearly the relative effectiveness of these pigments for skin and even yolk pigmentation of poultry.

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