Enrichment of Eggs with Lutein

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ABSTRACT Lutein is being considered as a nutrient for prevention of macular degeneration in the aging population. Two experiments were designed to study the transfer efficiency of lutein from the layers' diet into the egg. In experiment 1, laying hens were fed corn-soy diets supplemented with 0, 125, 250, 375, 500, 625, 750, or 1,000 ppm of lutein. After 30 d, eggs were collected and assayed for lutein. In a second study, layers were fed corn-soy diets or diets containing corn gluten meal and alfalfa, with or without added flaxseed. Diets in experiment 2 were supplemented with 0, 125, 250, or 500 ppm of lutein. Adding lutein to the layers' diet resulted in a significant ($P < 0.01$) increase in Roche color score of yolk within 7 d of supplementation. In experiment 1, lutein was transferred into the yolk ($P < 0.01$) increasing from a basal level of 0.3 mg to 1.5 mg/60 g of egg. However, there was no significant ($P > 0.05$) increase in yolk lutein with diet supplements >375 ppm. In the second experiment, using corn gluten meal and alfalfa further increased lutein content that leveled off at 2.2 mg/60 g of egg with a diet supplement of 500 ppm of lutein. Adding flax to these diets seemed to depress yolk lutein content. Yolk lutein content can be increased, although further studies are needed to investigate the major decline in transfer efficiency seen with higher levels of dietary supplementation.

(*Key words*: egg composition, lutein, macular degeneration)

2004 Poultry Science 83:1709–1712

INTRODUCTION

Carotenoids have been used for many years in the poultry industry as a means of pigmenting eggs and meat (Leeson and Summers, 1997). Their spectral qualities result in change in color of fat depots; depending on the actual xanthophyll pigment and the concentration in the birds' diet, they impart colors ranging from yellow to intense orange. Over the last 10 yr there has been increased awareness of the role of xanthophylls in human health, and in particular the roles of lutein and zeaxanthin in prevention of certain eye disorders.

Macular degeneration is the leading cause of blindness in developed countries, resulting in progressive and irreversible loss of central region vision. The most effective prevention to date is increasing our intake of lutein, which accumulates in the macular region of the eye and seems to aid in prevention of such blindness. Lutein and zeaxanthin are able to absorb blue light striking the retina, which is thought to initiate degeneration of the delicate surface membrane (Landrum and Bone, 2001). Lutein may also play a role as an antioxidant in macular surface membranes (Rapp et al., 2000). Lutein and zeaxanthin seem to be preferentially deposited in the retina, unlike β-carotene, which shows limited accumulation in the retina, despite being the most common xanthophyll pigment in our diets (Landrum and Bone, 2001).

Landrum et al. (1997) showed that the optical density of the macular pigment increased by 30% in humans supplemented with lutein, which equates to a 40% reduction in blue light reaching the retina. Moeller et al. (2000) suggested that xanthophyll intake might also influence development of cataracts. Eggs, although not normally the richest source of pigments, contain highly available and stable pigments that could be of importance in preventing cataract and macular degeneration. Landrum and Bone (2001) suggested the intake of lutein and zeaxanthin in North Americans is less than 1 mg/d, which is much less than the preventive levels being tested for these nutrients (Grando et al., 2003).

Eggs normally contain 0.3 to 0.5 mg of total xanthophylls, with just over half present as lutein (Steinberg et al., 2000). As with most fats and fat-soluble compounds, the composition of the egg is responsive to manipulation of such nutrients in the layer diet. There is limited information available on the efficiency of transport of various xanthophylls into the egg and the factors that influence such deposition. Grashorn and Steinberg (2002) showed 40% conversion of supplemental canthaxanthin into eggs. However, in the same study, efficiency of lutein transfer was closer to 10% and seemed to decline with higher levels of supplements. With a view to developing lutein-enriched eggs, 2 studies were conducted to quantitate the efficiency of transfer of lutein from feed into eggs.

Received for publication May 26, 2004.
Accepted for publication June 15, 2004.
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TABLE 1. Percentage diet composition (experiment 1)

Ingredients	Composition (%)
Corn	54.65
Wheat shorts	8.80
Soybean meal	18.00
Meat meal	5.00
Animal/vegetable fat	2.00
Limestone	9.50
Dicalcium phosphate	0.70
Salt	0.25
DL-Methionine	0.10
Vitamin-mineral premix	1.00
Calculated analysis	
ME (kcal/kg)	2,807
Crude protein (%)	17.40
Calcium (%)	4.18
Available P (%)	0.39
Methionine (%)	0.39
Met + Cys (%)	0.64
Lysine (%)	0.92

TABLE 2. Percentage diet composition (experiment 2)

	Diet 1	Diet 2	Diet 3
Corn	60.30	40.14	52.20
Wheat shorts		18.60	1.90
Soybean meal	25.70	15.50	15.10
Corn gluten meal		8.00	8.00
			8.00
Flaxseed (ground)			8.00
Animal/vegetable fat	1.60		
Corn oil		3.00	
DL-Methionine	0.14	0.08	0.07
L-Lysine		0.07	0.09
Salt	0.34	0.30	0.30
Limestone	9.50	9.48	9.40
Dicalcium phosphate	1.42	1.33	1.44
Vitamin-mineral premix	1.00	1.00	1.00
Alfalfa meal		2.50	2.50
Calculated analysis			
ME (kcal/kg)	2,800	2,800	2,800
Crude protein (%)	17.5	18.9	19.0
Calcium (%)	4.0	4.0	4.0
Available P (%)	0.45	0.45	0.45
Methionine (%)	0.44	0.44	0.44
Met + Cys (%)	0.71	0.72	0.73
Lysine (%)	0.94	0.86	0.86

MATERIALS AND METHODS

Experiment 1

Corn-soy diets were formulated appropriate for layers at 30 to 35 wk of age (Table 1). Graded levels of lutein were added to this diet, in the form of a 5% tagets premix (the most commonly used form in the human food and pharmaceutical industries) as provided by Roche. Diet inclusions provided calculated lutein levels of 125, 250, 375, 500, 625, 750, and 1,000 ppm. Each of the diets was fed to 6 replicate individually caged Shaver White SCWL layers of a commercial strain. The birds were 32 wk old at the start of the feeding study. On d 7, 14, 21, and 28, all eggs were collected and broken out for assessment of yolk color using the subjective Roche color scale of 1 to 15. On d 29 or 30, one egg was collected from each replicate bird and was used for assay of lutein and zeaxanthin.

Experiment 2

Three basal diets were formulated to provide similar levels of nutrients (Table 2). Diet 1 was a corn-soy basal diet comparable to that used in experiment 1. Diet 2 included corn gluten meal and alfalfa as well as corn oil rather than animal/vegetable fat. These ingredients were used to elevate the natural xanthophyll content of the diet and potentially increase efficiency of transfer of lutein into eggs. Diet 3 contained ground flaxseed, because it is realized that commercial application of lutein-enriched eggs may well be in conjunction with other nutraceuticals such as omega-3 fats. Each diet was fed to 12 replicate individually caged SCWL layers that were 55 wk old at the start of the experiment. Egg production was monitored continuously; egg weight, shell deformation, and albumen height were measured from one egg per bird collected on d 50 to 51. From d 52 to 55, one egg was collected from each replicate hen, and these were stored at 4°C before subsequent assay for lutein and zeaxanthin.

Egg Analyses

Yolks were separated from albumen and approximately 500 mg of yolk added to 1 g of glass beads in a scintillation vial. Five milliliters of acetone was added and the tubes were vortexed for 20 s. After setting for 1 h, 1 mL of the extract was placed in HPLC vials and the acetone was evaporated under gentle heat. The residue was dissolved in 1 mL of hexane:ethyl acetate (65:35), and the tubes were vortexed again. For quantification, lutein and zeaxanthin were passed through a 250 mm × 4.6 m nitrite banded spherisorb column using 5 μm particles. A calibration curve was prepared using lutein and zeaxanthin standards.

RESULTS AND DISCUSSION

The assayed lutein content of the various experimental diets agreed reasonably well with calculated values (Tables 3 and 4). Diet had no effect on egg production, egg weight, feed intake, or shell quality ($P > 0.05$). In experiment 1, as the lutein content of the diet increased, there was an increase ($P > 0.01$) in lutein content of the eggs (Table 3). However, the efficiency of transfer from feed to eggs diminished quickly as the inclusion level of lutein increased. The most noticeable enrichment occurred with the initial addition of 125 ppm of lutein to the diet. The highest level of enrichment was achieved with 500 ppm of dietary lutein, although there was no difference in egg lutein content for dietary lutein additions of 375 to 1,000 ppm ($P > 0.05$). There was a dramatic increase in egg yolk color as lutein was added to the diet, and this was little affected by diet level of lutein (Figure 1). Within just 7 d of supplementation, egg yolk color increased from 6/7 to 12/13 on the Roche scale. Egg yolk color leveled off at around 13/14 and was unaffected by dietary lutein supplements above 250 ppm.

TABLE 3. Lutein and zeaxanthin content of eggs (experiment 1)

Dietary lutein (ppm)				
Expected	Assayed	Lutein	Zeaxanthin	Lutein + zeaxanthin
		mg/60 g of egg		
0	4.9	0.16[d]	0.16[a]	0.32[c]
125	126	1.17[c]	0.16[a]	1.33[b]
250	250	1.25[bc]	0.14[ab]	1.39[b]
375	318	1.37[abc]	0.14[abc]	1.50[ab]
500	451	1.49[ab]	0.13[bc]	1.62[ab]
625	588	1.45[ab]	0.12[bc]	1.57[ab]
750	733	1.49[ab]	0.11[c]	1.60[ab]
1,000	1,091	1.62[a]	0.12[bc]	1.74[a]
SD		0.15	0.02	0.16
Significance		**	**	**

[a–c]Means within columns with different superscripts are significantly different.

**$P < 0.01$.

In experiment 2, lutein was again transferred into the egg yolk (Table 4). The use of corn gluten meal and alfalfa gave a boost to lutein accumulation in the egg, whereas inclusion of flaxseed generally resulted in less bioaccumulation in the egg. The highest level of yolk enrichment was seen in birds fed corn gluten meal plus alfalfa with 500 ppm of lutein added to the diet. However, the efficiency of transfer of lutein into the yolk was very low with the higher supplements of dietary lutein. Assuming a feed intake of around 95 g/bird per d with about 50 g of daily egg mass production, the conversion efficiency of lutein from feed to eggs was around 10% with 125 ppm in the diet, declining to 2 to 3% with a supplement level of 500 ppm. Dietary lutein supplementation had no effect ($P > 0.05$) on egg production, egg weight, or any other egg characteristics measured.

The results from these experiments indicate that lutein is transferred into the yolk, although transfer efficiency is very low, especially at higher levels of diet inclusion. Steinberg et al. (2000) showed a similar trend of declining efficiency of lutein transfer into eggs with increased levels in the diet, although in this study, the maximum dietary level was only 120 ppm. In a subsequent study, Grashorn and Steinberg (2002) showed much higher transfer efficiency for canthaxanthin, although dietary levels were more moderate than those used in the current study. The level of lutein in human diets necessary to prevent macular degeneration has not been clearly defined, with recommendations ranging from 10 to 20 mg/d. Much of the classical work with lutein and optical density of the macular pigment involved daily intake of up to 30 mg/d. Because average daily intake of lutein in adults seems to be less than 1 mg/d (Landrum and Bone, 2001), enrichment of eggs with 1.5 to 2 mg, as achieved here, represents a significant contribution to our diet. As shown in Figure 1, yolk color changes rapidly and dramatically in response to feeding lutein. The fact that yolk color changed within just 7 d was somewhat surprising. A yolk grows in mass over a 14 to 20 d period, and so it was expected that yolk color would not peak until 14 to 21 d following diet supplementation. However, 'observable' color is essentially controlled by color of the outer yolk depots, and

TABLE 4. Lutein and zeaxanthin content of eggs (experiment 2)

Diet type[1]	Dietary lutein (ppm)		Lutein	Zeaxanthin	Lutein + zeaxanthin
	Expected	Assayed		(mg/60 g of egg)	
Corn-soy	0	5	0.18[d]	0.11[c]	0.29[d]
Corn-soy	125	128	1.43[bc]	0.20[a]	1.63[bc]
Corn-soy	250	237	1.52[bc]	0.18[ab]	1.71[bc]
Corn-soy	500	518	1.65[b]	0.14[bc]	1.79[bc]
Corn-soy, CGM, ALF	0	22	0.29[d]	0.19[ab]	0.48[d]
Corn-soy, CGM, ALF	125	136	1.38[bc]	0.22[a]	1.59[bc]
Corn-soy, CGM, ALF	250	247	1.72[ab]	0.22[a]	1.94[ab]
Corn-soy, CGM, ALF	500	496	2.04[a]	0.20[a]	2.24[a]
Corn-soy, CGM, ALF, Flax	0	24	0.24[d]	0.17[ab]	0.41[d]
Corn-soy, CGM, ALF, Flax	125	118	1.23[c]	0.22[a]	1.45[c]
Corn-soy, CGM, ALF, Flax	250	241	1.40[bc]	0.20[a]	1.60[bc]
Corn-soy, CGM, ALF, Flax	500	518	1.39[bc]	0.17[ab]	1.57[bc]
SD			0.14	0.02	0.16
Significance			**	**	**

[a–c]Means within columns with different superscripts are significantly different.

**$P < 0.01$.

[1]CGM = corn gluten meal; ALF = alfalfa meal.

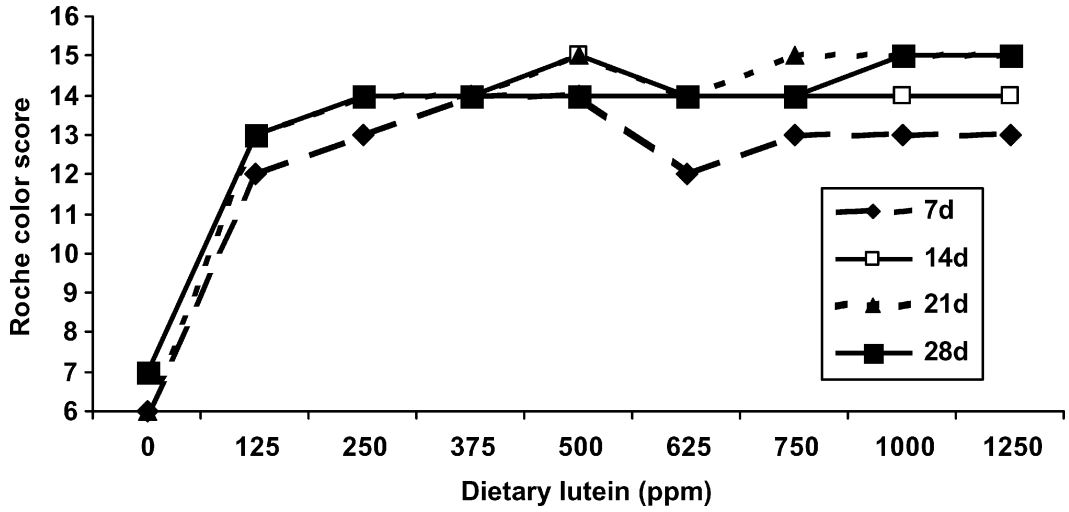

FIGURE 1. Effect of dietary lutein on Roche egg color.

these arise late in yolk growth. Consequently, even though yolk color changes quickly, we do not expect to see such rapid bioaccumulation with lutein. With omega-3 enriched eggs, it usually takes at least 21 d to ensure that all eggs are sufficiently enriched, and we expect the same situation to apply to deposition of yolk lutein over time.

The observation of reduced egg lutein content when flax was added to the diet is of some concern. Because it seems advantageous to market eggs containing a number of nutraceuticals, the inclusion of lutein combined with sources of omega-3 fats is a likely goal. The reason for interference with flax is unknown, although it may simply relate to the problems of indigestion often seen with this ingredient (Bean and Leeson, 2002).

This study has shown that it is possible to increase egg yolk lutein 5 to 8 times above regular concentrations, and that such enriched eggs will supply a meaningful contribution to our diet. Further increase in yolk lutein content may arise from studies that seek to optimize fat use in layers.

ACKNOWLEDGMENTS

This work was funded by the Ontario Ministry of Agriculture and Food, the Canadian Egg Marketing Agency, Egg Producers of Ontario, the National Sciences and Engineering Research Council of Canada, Roche Canada, and Kemin Industries. The authors would like to thank Richard Snyder, Kemin Industries, and Alfred Blanch, Roche Vitamins, Ltd. for assistance with lutein assays.

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