

# Effects of Canthaxanthin on Egg Production, Egg Quality, and Egg Yolk Color in Laying Hens

J. H. Cho<sup>1</sup>, Z. F. Zhang<sup>1</sup> & I. H. Kim<sup>1</sup>

<sup>1</sup> Department of Animal Resource & Science, Dankook University, #29 Anseodong, Cheonan, Choongnam, Korea

Correspondence: I. H. Kim, Department of Animal Resource & Science, Dankook University, #29 Anseodong, Cheonan, Choongnam, Korea. Tel: 82-41-550-3652. E-mail: inhokim@dankook.ac.kr

Received: October 9, 2012 Accepted: October 25, 2012 Online Published: December 14, 2012

doi:10.5539/jas.v5n1p269

URL: <http://dx.doi.org/10.5539/jas.v5n1p269>

## Abstract

The effects of canthaxanthin (CTX) on egg production, (average daily feed intake) ADFI, egg quality, and yolk color compared with natural xanthophylls in feedstuffs in laying hens were studied in a 5-wk trial. A total of 280 36-wk-old ISA Brown layers were divided into the following 7 treatments: T1, negative control (30% corn+20% wheat); T2, diet with 50% corn; T3, diet with 5% DDGS; T4, diet with 10% DDGS; T5, T1+0.011% CTX; T6, T1+0.021% CTX; T7, commercial diet with 40% corn. Although ADFI, and egg quality were not affected by dietary treatments, the egg production was higher ( $P<0.05$ ) in diet supplemented with 0.021% colorant than diet with 50% corn, and diet supplemented 0.011% colorant. Yolk color in layers fed the diet contained 10% DDGS was higher ( $P<0.05$ ) than the layers fed the T1 diet, and the diet with 5% DDGS. Yolk color score was linearly improved ( $P<0.05$ ) when the diet supplemented with 0.011%, and 0.021% CTX. Higher yolk color score was also observed ( $P<0.05$ ) when layers fed commercial diet compared with control diet, and the diet with higher natural xanthophylls. In conclusion, egg production and yolk color were improved when layers fed diet supplemented with colorant.

**Keywords:** canthaxanthin, egg production, egg quality, egg yolk color, laying hens

## 1. Introduction

Egg yolk color is a major concern to consumers and greatly affects their purchasing behavior (De-Groote, 1970; Fletcher, 1999). Although xanthophylls containing diet does not provide higher nutrition levels, enhancements are observed in pigmentation of egg yolk (Bortolotti et al., 2003; Na et al., 2004; Wang et al., 2007).

High-energy concentrated feeds having low pigment content, which must be supplemented with pigments, are usually used industrially (Vargas et al., 1998). Dried distillers' grains (DDGS) is one of the most important ingredients of laying hen feed contains lutein, zeaxanthin, which are the main xanthophylls in egg yolks (Karunajeeva et al., 1984; Bailey & Chen, 1989). The activity of the xanthophylls is limited by their relative instability to high temperature and light. However, Williams et al. (1963) reported that natural sources of such pigments were more efficiently used than were the purified forms.

About 6-8 mg of xanthophylls/kg of feed is required to produce an egg color that is acceptable to consumers (Avila et al., 1990). Even this small quantity of pigment represents a considerable cost for egg producers (Williams, 1992). Several researches have been carried out to seek improvements of pigmentation efficiency (Middendorf et al., 1980; Marusich & Baurenfeind, 1981). The synthetic pigment canthaxanthin (CTX) is found 1.5-5 times as potent as natural xanthophylls for the pigmentation of egg yolk (Akiba et al., 2000). Presently, one type of chemically isomerized marigold with 10% of xanthophylls as CTX in practical layer diet (maize-wheat-soybean) was supplemented to evaluate the effects of CTX on egg production, egg quality and egg-yolk color, compared to a diet containing a high content of xanthophyll.

## 2. Materials and Methods

All birds used in this trial were handled in accordance with guidelines set forth by the Dankook University Committee on Laboratory Animal Care.

### 2.1 Experimental Design, Animals, and Diets

A total of 280 ISA Brown laying hens were randomly assigned to 1 of 7 dietary treatments in an experiment that was conducted with hens from 36 to 40 wk of age. Dietary treatments were: T1, negative control (30% corn+20% wheat); T2, diet with 50% corn; T3, diet with 5% DDGS; T4, diet with 10% DDGS; T5, T1+0.011% CTX; T6, T1+0.021% CTX (Synthetic canthaxanthin beadlets (CarophyllRed, Lisbon, Portugal) with 10% canthaxanthin); T7, commercial diet with 40% corn. Hens were weighed individually at the onset of the experiment and then assigned to treatments in a randomized block design based on house location. There were 4 replicates for each treatment, with 5 adjacent cages (2 hens/cage, 38.1-cm width × 50-cm length × 40-cm height) representing a replicate. The hens were housed in a windowless and temperature controlled room that was maintained at 20°C with a daily lighting schedule of 17L:7D. There was 1 empty cage between every 5 cages. Hens were allowed to water and feed *ad libitum* through the nipple of an automatic drinker and a common trough feeder, respectively. The experimental diets (Table 1) were formulated in accordance with recommendations in the breeder's manual for ISA Brown hens and to meet the nutrient recommendations (NRC, 1994), and were provided in mesh form. Dietary calcium (Ca), phosphorus (P), crude protein, lysine and methionine were analysed according to the procedures described by the AOAC (1995). Dietary Ca was assayed by atomic absorption spectrophotometry after wet ash procedures and P was determined by colorimetry. Lysine content was measured using an amino acid analyzer (Beckman 6300, Beckman Coulter, Inc., Fullerton, California, U.S.A.) after 24-h 6 N-HCl hydrolysis at 110°C (AOAC, 1995). Gross energy was determined by using a Parr 6100 oxygen bomb calorimeter (Parr instrument Co., Moline, Illinois, U.S.A.). Before the beginning of the experiment, 29-wk-old hens were purchased and provided with a basal diet for a 7-wk adjustment period, and birds not laying well were replaced.

Table 1. Compositions of experimental diets

Items	T1	T2	T3	T4	T5	T6	T7
Ingredients, %							
Corn	30.00	50.00	30.00	30.00	30.00	30.00	40.00
Wheat	20.00	-	15.00	10.00	20.00	20.00	10.00
Grain sorghum	10.00	10.00	10.00	10.00	10.00	10.00	3.00
Soybean meal (CP 46%)	19.20	21.20	20.70	20.20	19.19	19.18	19.20
Wheat bran	7.00	5.00	5.00	5.00	7.00	7.00	7.00
DDGS	-	-	5.00	10.00	-	-	7.00
Tallow	4.40	4.40	4.90	5.40	4.40	4.40	4.40
Dicalcium phosphate	1.30	1.30	1.30	1.30	1.30	1.30	1.30
Limestone	7.50	7.50	7.50	7.50	7.50	7.50	7.50
Salt	0.30	0.30	0.30	0.30	0.30	0.30	0.30
DL-Methionine (50%)	0.10	0.10	0.10	0.10	0.10	0.10	0.10
Vitamin premix <sup>2</sup>	0.10	0.10	0.10	0.10	0.10	0.10	0.10
Trace mineral premix <sup>3</sup>	0.10	0.10	0.10	0.10	0.10	0.10	0.10
Colorant					0.011	0.021	
Calculated value, %							
AME, kcal/kg	2,934	2,956	2,979	2,975	2,940	2,938	2,904
CP	15.60	15.65	15.65	15.65	15.65	15.65	15.65
Lys	0.83	0.85	0.84	0.85	0.84	0.84	0.82
Met+Cys	0.94	0.93	0.93	0.92	0.92	0.92	0.93
Ca	3.35	3.40	3.40	3.40	3.37	3.37	3.40
P	0.65	0.67	0.66	0.66	0.66	0.66	0.65
Chemical analysis value, %							
AME, kcal/kg	2,945	2,966	2,960	2,947	2,955	2,950	2,900
Crude protein	15.69	15.70	15.65	15.70	15.70	15.68	15.68
Lys	0.92	0.93	0.91	0.90	0.91	0.90	0.91
Ca	3.43	3.50	3.47	3.48	3.45	3.44	3.50
P	0.66	0.67	0.65	0.66	0.64	0.62	0.66

T1, negative control (30% corn+20% wheat); T2, positive control (50% corn); T3, 5% DDGS; T4, 10% DDGS; T5, T1+0.011% CTX; T6, T1+0.021% CTX; T7, commercial feed (40% corn). Provided per kilogram of diet is 125,000 IU of vitamin A; 2,500 IU of vitamin D<sub>3</sub>; 10 mg of vitamin E; 2 mg of vitamin K<sub>3</sub>; 1 mg of vitamin B<sub>1</sub>; 5 mg of vitamin B<sub>2</sub>; 1 mg of vitamin B<sub>6</sub>; 15 mg of vitamin B<sub>12</sub>; 500 mg of folic acid; 35,000 mg of niacin; 10,000 mg of Ca-pantothenate; and 50 mg of biotin. Provided per kilogram of diet is 8 mg of Mn (as MnO<sub>2</sub>); 60 mg of Zn (as ZnSO<sub>4</sub>); 5 mg of Cu (as CuSO<sub>4</sub>·5H<sub>2</sub>O); 40 mg of Fe (as FeSO<sub>4</sub>·7H<sub>2</sub>O); 0.3 mg of Co (as CoSO<sub>4</sub>·5H<sub>2</sub>O); 1.5 mg of I (as KI); and 0.15 mg of Se (as Na<sub>2</sub>SeO<sub>3</sub>·5H<sub>2</sub>O).

## 2.2 Sampling and Measurements

Daily records of egg production and weekly records of feed consumption were maintained. Egg production was expressed as average hen day production. A total of 20 saleable eggs (no shell defects, cracks, or double yolks) were randomly collected at 17:00 pm from each treatment (5 per replicate,  $n=20$ ) on a weekly basis and used to determine the egg quality at 20:00 pm the same day. The specific gravity of eggs was determined by using the saline flotation method of Hempe et al. (1998). Salt solutions were made in incremental concentrations of 0.005 in the range from 1.065 to 1.120. Eggshell breaking strength was evaluated by using an eggshell force gauge (Robotmation. Co., Ltd., Japan). Eggshell thickness was measured on the large end, equatorial region, and small end, respectively, using a dial pipe gauge (Ozaki MFG. Co., Ltd., Japan). Finally, egg weight, egg yolk color, and Haugh units (HU) were evaluated using an egg multimeter (Touhoku Rhythm. Co., Ltd., Japan).

## 2.3 Statistical Analysis

All data were subjected to the statistical analysis as a randomized complete block design using the GLM procedures (SAS Inst. Inc., Cary, NC). The initial BW was used as a covariate for ADFI. Differences among treatment means were determined using the Duncan's multiple range test, with a  $P<0.05$  indicating a significance.

## 3. Results

### 3.1 Egg Production

The egg production was higher ( $P<0.05$ ) in T6 treatment (T1+0.021% CTX) than that of T2 (50% corn) and T5 (T1+0.011% CTX) treatments (Table 2).

Table 2. Effect of dietary raw material and colorant on egg production in layers

Items, %	T1	T2	T3	T4	T5	T6	T7	T1	P value
Egg production	98.6 <sup>ab</sup>	97.4 <sup>b</sup>	98.6 <sup>ab</sup>	97.9 <sup>ab</sup>	97.4 <sup>b</sup>	99.0 <sup>a</sup>	98.2 <sup>ab</sup>	0.4	0.035

T1, negative control (30% corn+20% wheat); T2, positive control (50% corn); T3, 5% DDGS; T4, 10% DDGS; T5, T1+0.011% CTX; T6, T1+0.021% CTX; T7, commercial feed (40% corn). SE, Standard error. Treatment values followed by same alphabet do not differ significantly at  $P=0.05$ .

### 3.2 ADFI

There's no difference ( $P>0.05$ ) was observed in ADFI by dietary treatments (Table 3).

Table 3. Effect of dietary raw material and colorant on ADFI in layers

Items	T1	T2	T3	T4	T5	T6	T7	SE	P value
ADFI, g	135	137	135	136	135	136	135	1	0.657

T1, negative control (30% corn+20% wheat); T2, positive control (50% corn); T3, 5% DDGS; T4, 10% DDGS; T5, T1+0.011% CTX; T6, T1+0.021% CTX; T7, commercial feed (40% corn). SE, Standard error.

### 3.3 Egg Quality

During the experiment, no effects ( $P>0.05$ ) of dietary treatments were observed on egg gravity, strength, yolk height, shell color, haugh unit, and shell thickness (Table 4).

Table 4. Effect of dietary raw material and colorant on egg quality in layers

Items	T1	T2	T3	T4	T5	T6	T7	SE	P value
Gravity	1.10	1.10	1.10	1.10	1.10	1.10	1.10	0.00	0.722
Strength (kg/cm <sup>2</sup> )	3.88	3.85	3.97	3.85	3.83	3.81	3.84	0.05	0.214
Weight (g)	60.3	60.8	60.6	61.0	60.5	60.6	60.5	0.2	0.325
Height (mm)	8.2	8.1	8.1	8.0	8.0	8.1	8.2	0.1	0.841
Shell color	11.10	11.5	11.2	11.5	11.2	11.3	11.2	0.2	0.651
Haugh unit	90.1	89.0	89.6	88.9	89.1	89.5	89.8	0.4	0.252
Shell thickness (mm)	39.7	39.3	39.8	38.6	39.2	38.6	39.7	0.5	0.698

T1, negative control (30% corn+20% wheat); T2, positive control (50% corn); T3, 5% DDGS; T4, 10% DDGS; T5, T1+0.011% CTX; T6, T1+0.021% CTX; T7, commercial feed (40% corn). SE, Standard error.

Table 5. Effect of dietary raw material and colorant on yolk color in layers

Items	T1	T2	T3	T4	T5	T6	T7	SE	P value
Yolk color	4.8 <sup>c</sup>	5.4 <sup>de</sup>	5.0 <sup>c</sup>	5.9 <sup>d</sup>	7.6 <sup>b</sup>	9.7 <sup>a</sup>	6.5 <sup>c</sup>	0.19	0.024

T1, negative control (30% corn+20% wheat); T2, positive control (50% corn); T3, 5% DDGS; T4, 10% DDGS; T5, T1+0.011% CTX; T6, T1+0.021% CTX; T7, commercial feed (40% corn). SE, Standard error.

### 3.4 Yolk Color

Laying hens fed the T6 diet had the highest ( $P<0.05$ ) yolk color among dietary treatments, while yolk color in T4 treatment was higher ( $P<0.05$ ) than those in T1 and T3 treatments. Yolk color score was increased ( $P<0.05$ ) when the diet supplemented with colorant compared to other diets, and the effect was improved ( $P<0.05$ ) by increasing the content of the colorant. Higher ( $P<0.05$ ) yolk color score was also observed when layers fed commercial diet compared to T1, T2, T3, and T4 treatments.

## 4. Discussion

The present study demonstrates that the dietary raw material and CTX had no effects on feed consumption and egg quality characteristics such as egg gravity, shell-breaking strength, shell thickness, and Haugh units. These data corroborate the results obtained with no feed consumption (Khaton et al., 1999; Hasin et al., 2006), egg gravity (Saha et al., 1999; Hasin et al., 2006) in hens fed a diet enriched in CTX.

Presently, egg production was higher ( $P<0.05$ ) in a diet with 0.021% CTX than a diet with 50% corn, and a diet supplemented with 0.011% CTX. These results differ from the previous findings of no effect of colorant on egg production (Garcia et al., 2002; Soto-Salanova, 2003; Kanda et al., 2011).

Pigmentation of egg yolks is influenced mostly by layer diet (Colin et al., 2004) and DDGS is a rich source of xanthophylls (Bailey & Chen, 1989). Natural xanthophyll is well-absorbed by hen intestinal cells (Gouveia et al., 1996) and is transferred to the yolk (Donald & William, 2002) after being released into the circulatory system (Salma et al., 2007). However, in natural products, xanthophylls are unstable and effective levels may decline as a result of oxidation during prolonged storage. Previous studies have also reported that different forms of xanthophylls have different deposition rate in eggs (Bowen et al., 2002). The discussion continues about whether free or esterified xanthophylls have higher bioavailability, not only with respect to animal feed, but also in view of human dietary supplements that contain free or esterified forms of lutein (Bowen et al., 2002). The zeaxanthin content of corn deposited in the yolk can be 7%, while the content of some synthetic products such as  $\beta$ -apo-8-carotenoic acid ethyl ester and CTX can reach 34% (Roche Vitamins & Fine Chemicals, 1988).

In our study, we found that yolk color was enhanced by a diet containing 10% DDGS, which was agree with an earlier study reported that intact yolk color was enhanced quickly with a diet containing 10% DDGS after about 2 months when 5% DDGS was fed to birds previously fed a corn-soybean meal with no additional pigments

(Roberson et al., 2005). Also, the present observation that yolk color scores linearly increased with an increase in the concentration of dietary CTX (0.011%-0.021%) in laying hens fed a low-carotenoid diet is consistent with the finding that the yolk color score increased with increasing percentages of dietary paprika (Niu et al., 2008).

This research demonstrated that CTX could be incorporated into egg yolks at 0.011% and 0.021%, and that a diet with 10% DDGS also improves egg yolk color compared with a diet low in xanthophylls.

## 5. Conclusion

In conclusion, it can be concluded that dietary CTX supplementation at level of 0.021% can affect egg production and improve the pigmentation of egg yolk. However, it is still necessary to conduct more researches of CTX in laying hens, due to the limited data on the mechanism of pigmentation in layers.

## Acknowledgment

The authors would like to acknowledge the financial assistance (No. PJ0081212012 of Bio-Green 21) provided by the Rural Development Administration of Korea.

## References

- Akiba, Y., Sato, K., Takahashi, K., Takahashi, Y., Furuki, A., Konashi, S., ... Nagao, H. (2000). Pigmentation of egg yolk with yeast *phaffia rhodozyma* containing high concentration of astaxanthin in laying hens fed on a low-carotenoid diet. *J. Poultry. Sci.*, *3*, 77-85.
- Avila, E., Shimada, A., Llamas, S., & Otros aditivos, G. (1990). In *Anabólicos y Aditivos en la Producción Pecuaria*, 1st ed. *Consultores en Producción Animal, S.C., Me'xico*, 239-250.
- Bailey, C. A., & Chen, B. H. (1989). Chromatographic analyses of xanthophyl in egg yolks from laying hens fed Turf Bermudagrass (*Cynodon dactylon*) meal. *J. Food. Sci.*, *54*, 584-586. <http://dx.doi.org/10.1111/j.1365-2621.1989.tb04658.x>
- Bortolotti, G. R., Negro, J. J., Surai, P. F., & Prieto, P. (2003). Carotenoids in eggs and plasma of red-legged partridges: effects of diet and reproductive output. *Physiol. Biochem. Zool.*, *76*, 367-374. <http://dx.doi.org/10.1086/375432>
- Bowen, P. E., Herbst-Espinosa, S. M., Hussain, E. A., & Stacewicz-Sapuntzakis, M. (2002). Esterification does not impair lutein bioavailability in humans. *J. Nutr.*, *132*, 3668-3673.
- Colin, G. S., George, B., & Ensminger, M. E. (2004). *Poultry Science* (4th ed). New Jersey: Pearson Prentice Hall, Upper Saddle River.
- De-Groote, G. (1970). Research on egg yolk pigmentation and its practical application. *World's Poult. Sci. J.*, *20*, 435-441. <http://dx.doi.org/10.1079/WPS19700005>
- Donald, D. B., & William, D. W. (2002). *Commercial Chicken Meat and Egg production* (5th ed). USA: Kluwer Academic Publishers.
- Fletcher, D. L. (1999). Broiler breast meat color variation, pH, and texture. *Poult. Sci.*, *78*, 1323-1327.
- Garcia, E. A., Mendes, A. A., Pizzolante, C. C., Goncalves, H. C., Oliveira, R. P., & Silva, M. A. (2002). Effect of canthaxanthin levels on performance and egg quality of laying hens. *Braz. J. Poultry. Sci.*, *4*, 1-4.
- Gouveia, L., Veloso, V., Reis, A., Fernandes, H., Novais, J., & Empis, J. (1996). *Chlorella Vulgaris* used to colour egg yolk. *J. Sci. Food. Agr.*, *70*, 167-172. [http://dx.doi.org/10.1002/\(SICI\)1097-0010\(199602\)70:2<167::AID-JSFA472>3.0.CO;2-2](http://dx.doi.org/10.1002/(SICI)1097-0010(199602)70:2<167::AID-JSFA472>3.0.CO;2-2)
- Hasin, B. M., Ferdous, A. J. M., Islam, M. A., Uddin, M. J., & Islam, M. S. (2006). Marigold and orange skin as egg yolk color promoting agents. *Int. J. Poultry. Sci.*, *5*, 979-987. <http://dx.doi.org/10.3923/ijps.2006.979.987>
- Hempe, J. M., Lauxen, R. C., & Savage, J. E. (1998). Rapid determination of egg weight and specific gravity using a computerized data collection system. *Poult. Sci.*, *67*, 902-907. <http://dx.doi.org/10.3382/ps.0670902>
- Kanda, L., Koh-en, Y., Tsutomu, K., & Keiko, S. (2001). Enhancement of yolk color in raw and boiled egg yolk with lutein from marigold flower meal and marigold flower extract. *J. Poultry. sci.*, *48*, 25-32.
- Karunajeewa, H., Hughes, R. S., McDonald, M. W., & Shenstone, F. S. (1984). A review of factors influencing pigmentation of egg yolks. *World's Poult. Sci. J.*, *40*, 52-65. <http://dx.doi.org/10.1079/WPS19840006>
- Karunajeewa, H. (1980). The deposition of synthetic oxycarotenoids in egg yolks. *World. Poult. Sci. J.*, *36*, 219-222. <http://dx.doi.org/10.1079/WPS19800011>

- Khaton, A., Ali, M. A., & Dingel, J. G. (1999). Comparison of the nutritive value for laying hens of diets containing azolla (*Azolla pinata*) based on formulation using digestible protein and digestible amino acid. *Anim. Feed Sci. Technol.*, *81*, 43-56. [http://dx.doi.org/10.1016/S0377-8401\(99\)00071-1](http://dx.doi.org/10.1016/S0377-8401(99)00071-1)
- Marusich, W. L., & Baurenfeind, J. C. (1981). Oxycarotenoids in poultry feeds. In J. C. Baurenfeind (Ed.), *Carotenoids as Colorants and Vitamin A Precursors* (pp. 319-462). New York: Academic Press.
- Middendorf, D. F., Childs, G. R., & Cravens, W. W. (1980). Variation in the biological availability of xanthophyll within and among generic sources. *Poult. Sci.*, *59*, 1460-1470. <http://dx.doi.org/10.3382/ps.0591460>
- Na, J. C., Song, J. Y., Lee, B. D., Lee, S. J., Lee, C. Y., & An, G. H. (2004). Effect of polarity on absorption and accumulation of carotenoids by laying hens. *Anim. Feed. Sci. Technol.*, *117*, 305-315. <http://dx.doi.org/10.1016/j.anifeedsci.2004.08.012>
- Niu, Z., Fu, J., Gao, Y., & Liu, F. (2008). Influence of paprika extract supplement on egg quality of laying hens fed wheat-based diet. *Int. J. Poultry. Sci.*, *7*, 887-889. <http://dx.doi.org/10.3923/ijps.2008.887.889>
- NRC. (1994). *Nutrient Requirements of Poultry*. 9th rev. Ed. Washington DC: Natl. Acad. Press.
- Roberson, K. D., Kalbfleisch, J. L., Pan, W., & Charbeneau, R. A. (2005). Effect of corn distiller's dried grains with solubles at various levels on performance of laying hens and egg yolk color. *Int. J. Poultry. Sci.*, *4*, 44-51. <http://dx.doi.org/10.3923/ijps.2005.44.51>
- Roche vitamins and fine chemicals. (1988). Egg yolk pigmentation with carophyll (3rd ed) (p. 1218). Switzerland: Hoffmann-La Roche. Ltd., Basel.
- Saha, P. K., Chowdhury, S. D., & Saha, S. K. (1999). Replacement value of two Bangladeshi varieties of yellow corn for wheat in the diet of laying chicken. *A. A. J. Ani. Sci.*, *12*, 776-782.
- Salma, U. A., Miah, G., Tareq, K. M. A., Maki, T., & Tsujii, H. (2007). Effects of dietary *Rhodobacter capsulatus* on egg-yolk cholesterol and laying hen performance. *Poultry. Sci.*, *86*, 714-719.
- Soto-Salanova, M. F. (2003). Natural pigments: practical experiences. In P. C. Garnsworthy, J. Wiseman (Eds.), *Recent Advances in Animal Nutrition*. Nottingham. UK.: Nottingham University Press.
- Vargas, F. D., Lo'pez, O. P., & González, E. A. (1998). Effects of sunlight illumination of marigold flower meals on egg yolk pigmentation. *J. Agric. Food*, *46*, 698-706. <http://dx.doi.org/10.1021/jf9702454>
- Wang, Y. M., Connor, S. L., Wang, W., Johnson, E. J., & Connor, W. E. (2007). The selective retention of lutein, meso-zeaxanthin and zeaxanthin in the retina of chicks fed a xanthophylls-free diet. *Exp. Eye. Res.*, *84*, 591-598. <http://dx.doi.org/10.1016/j.exer.2006.11.013>
- Williams, W. D. (1992). Origin and impact of color on consumer preference for food. *J. Poultry. Sci.*, *71*, 744-746. <http://dx.doi.org/10.3382/ps.0710744>
- Williams, W. P., Davies, R. E., & Couch, J. R. (1963). The utilization of carotenoids by the hen and chick. *Poult. Sci.*, *42*, 691-699. <http://dx.doi.org/10.3382/ps.0420691>