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**feature ARTICLE**

## Investigating the Effect of Diet on Nutrient Concentration in Eggs: How Your Breakfast Might Be Healthier than You Think

—**Gwen Stewart** (Edited by Kaitlin Matthews)

I never thought too much about the color of egg yolks. When I was younger, my mother told me that the fresher an egg is, the bolder the yolk color. However, as I discovered with the assistance of a Summer Undergraduate Research Fellowship, the yellow color is actually due to the presence of two carotenoids—lutein and zeaxanthin.

Lutein and zeaxanthin are two nutrients that may help in the prevention of age-related macular degeneration (AMD) and other diseases. A disease of the eye, AMD is the leading cause of irreversible blindness in Americans over the age of 60 (Ribaya-Mercado et al. 2004). Research shows that when people consume lutein and zeaxanthin in the course of their diet or in the form of supplements, the concentration of these beneficial carotenoids increases in the blood and retina of most individuals (Ribaya-Mercado et al. 2004). In this study, my goal was to raise levels of lutein and zeaxanthin in egg yolk to optimize potential human absorption, and to measure this change both by looking at the egg yolk and evaluating it biochemically. Having lived on a free range chicken farm since the age of eleven, it was a natural decision for me to incorporate chickens into my research. Other studies concerning lutein and zeaxanthin have been performed at the University of New Hampshire in recent years, but mine was the first to involve chickens.

### **There's Something Besides Cholesterol in Egg Yolks?**

Lutein and zeaxanthin (L/Z) are members of a carotenoid family that is found in green leafy vegetables and eggs and is known for its health benefits. Because neither chickens nor humans have the ability to synthesize carotenoids, it is logical to conclude that the carotenoids found in human tissue and blood must be obtained through diet. L/Z are found in high concentrations in plants, however humans can't easily absorb L/Z from plants due to chemical interference (Handelman et al., 1999). Eggs have a relatively low quantity of L/Z compared to vegetables, but humans have an easier time absorbing it because of LZ's fat solubility in eggs (Leeson et al., 2004; Handelman et al., 1999). Consequently, eggs are a very important resource of L/Z for human consumption.

Eggs got a bad rap in recent decades because of the relatively high cholesterol levels found in the yolks (Kritchevsky, 2004). Because blood cholesterol is considered a contributing factor to atherosclerosis, or hardening of the arteries, people have been wary of consuming eggs, fearing a connection with heart disease (McIntosh, 2000). However, no dietary link has ever been established between egg consumption and heart

disease (Fernandez, 2006). In fact, people who eat less than one egg per week actually have higher blood cholesterol levels than those who eat more than four eggs per week (Kritchevsky, 2004). In addition, it has been shown that approximately 70% of people have blood cholesterol that does not change significantly even when the amount of dietary cholesterol varies. The 30% who do respond to varying amounts of dietary cholesterol generally show increases in both low-density lipoproteins (LDL or "bad" cholesterol) and high-density lipoproteins (HDL or "good" cholesterol), thus maintaining a healthy ratio (Herron et al. 2004).

As more positive news on eggs has been revealed, egg consumption has started to increase (McIntosh, 2000). Not only are eggs an excellent source of L/Z, but they are also a good source of choline, folate, riboflavin, selenium; vitamins B-12, A, K, and D, and protein. Choline, an important nutrient previously less common in the human diet, can actually improve memory performance. While the protein content of eggs is less than an equivalent serving of beef, the combination of amino acids (building blocks of protein) is ideal, making eggs a better source of protein (Herron et al. 2004). In addition to being packed with nutrients offering various health benefits, eggs are a relatively inexpensive food, making them an excellent and affordable part of a healthy diet.

### **Eggs = Disease Prevention?**

A previous UNH study investigating blood and retinal lutein concentration changes in women who consumed eggs suggests that increasing L/Z in the diet can increase retinal concentrations of L/Z (Gerwick, 2005). This and other evidence suggests that carotenoids may have an important role in protecting humans against disease, most notably, the prevention of age-related macular degeneration (AMD) (Ribaya-Mercado et al. 2004). While the exact role of L/Z in the prevention of the disease is still unknown, it has been speculated that lutein acts as an antioxidant. In this role, lutein may help protect the eye against oxidative stress caused by shortwave light, a type of blue light absorbed by the eye that can form free radicals that are very dangerous and damaging to the macula in the eye, contributing to its aging (Santosa et al. 2005).

A carotenoid-rich diet is an effective strategy for decreasing the risk of AMD (Mares et al., 2006), and for protecting against cataracts, cancer, heart disease, and stroke. This could be due to the antioxidant properties of carotenoids, which can prevent DNA mutation and subsequently cancer (Ribaya-Mercado et al. 2004). Interestingly enough, AMD has overlapping risk factors with cardiovascular disease (CVD); thus treatment of AMD could help lower the risk of CVD as well.

The problem that we may face in supplementing L/Z concentration in eggs is the effect on what is considered the normal yellow yolk color. Of the three most common yellow carotenoids, L/Z are the two that have a chemical structure that may confer health benefits, while a third yellow carotenoid, the apo-ester form, functions solely, yet effectively, as a pigment. Apo-ester has a color deposition rate of 50% in egg yolks, whereas three times as much L/Z must be present to achieve the same color (Beardsworth et al.). For years the pigmentation qualities of apo-ester have been used by the egg industry to produce an aesthetically pleasing yolk color, but this practice has started to change since people in the industry discovered the superior health benefits of L/Z.

Considering all of these factors, the question remains: If eggs are a bioavailable source of L/Z and a chicken obtains L/Z from dietary sources, can the L/Z content in the yolk be manipulated by supplementing the

chickens' diet? The objective of my study was to determine if L/Z yolk levels would increase as a result of such supplemental measures, whether that increase would be observable both biochemically and visually, and whether it would be dose dependent.

## The Experiment

In this study, eighteen 20-week-old Leghorn chickens were divided randomly into three groups of six, each of which was assigned a letter designation. Within the groups, eggs were collected from four chickens, leaving two chickens on reserve. Dr. Robert Taylor, professor of animal sciences, oversaw the animals' care. The protocol for the use of chickens was approved by the Institutional Animal Care and Use Committee (#06046).

Starting a week before the experiment, we fed the chickens Blue Seal's Layer Mash, which essentially is finely ground cornmeal feed produced and mixed with nutrients specifically designed to meet the needs of egg-producing chickens. This mash was also used as the base for the chicken feed we developed at the start of the experiment. Group A had no supplement added to their food and served as the control group; group B consumed a diet supplemented with 125 ppm of lutein; and group C consumed a diet supplemented with 250 ppm of lutein. Kemin Industries of Des Moines, IA donated Oroгло™, a xanthophyll supplement for animal consumption made from corn and marigolds. We added 10.06g of Oroгло™ to 112 lbs of feed to reach 125 ppm lutein for group B, and 20.12g of Oroгло™ to the same amount of feed to reach 250 ppm lutein for group C. The various supplement mixtures were stored in separately labeled containers and kept in a cool, dry place for the duration of the study.

I conducted my research over a four-week time period, collecting baseline data in week zero. During the course of the study, groups A, B, and C consumed their respective diets and eggs were collected for analysis twice a week. The egg yolks from the two collection days were pooled, thereby yielding one egg sample from each hen per week.

Lab raters analyzed yolk color according to the Roche Yolk Color Fan (RYCF), which ranges from a pale yellow to a dark orange. Observations were made in a dark room with the yolk placed in a small plastic dish directly under a 60-watt light bulb. The rater held the RYCF above the yolk and looked down upon the fan and yolk for optimal comparison. Two people rated every yolk in the study, without knowledge as to which chicken or what treatment group the yolk had come from. To accomplish this, each yolk was coded and the yolks were not decoded until the end of the study.



The Roche Yolk Color Fan is the industry standard for rating the color of egg yolks. The fan ranges from a pale yellow at rating 1 to a dark orange at rating 15 (Vuilleumier, 1969). Theoretically, to achieve a score of zero, there would be little to no yellow pigments in the yolk. To achieve orange-ish ratings above 13, red pigments, such as canthaxanthin, would be present in the yolk in addition to the yellow pigments (Hamilton, 1992). The ratings seen in this study were between 7 and 11.5.

The yolks were biochemically analyzed using high performance liquid chromatography (HPLC), which separates chemicals based on polarity and allows for quantification based on spectral analysis. Because L/Z travels with fats, I performed a saponification procedure on the yolks before HPLC analysis to release the fats, thereby releasing L/Z for extraction.

The concentration of L/Z was determined using an internal standard which was added during the extraction and an external standard which was added to the HPLC machine. The internal and external standards are chemicals that act like controls, enabling comparison of the yolk concentrations. The HPLC machine creates a graph showing the area of each of the compounds in relation to the others based on their polarity. The area is translated to concentration using the standard curve.

## **What Happened?**

My expectations for this experiment were that control group A would see no change in L/Z concentrations, while groups B and C would each see a noticeable change correlating with the degree of supplement added. However, lutein and zeaxanthin concentrations did not show significant change in any of the groups, with the exception of a large increase of zeaxanthin concentration in group B, a change outside of expected normal fluctuations.

The significant increase of zeaxanthin seen in group B may be explained by the fact that the supplement, Oroglo™, is made up of corncobs and marigolds. Corncobs and marigolds both contain L/Z, but marigolds have a higher ratio of zeaxanthin. This higher ratio could account for the significant zeaxanthin increase seen in my experiment. The ratio of lutein to zeaxanthin concentrations can be seen in [figure 1](#).

One reason for the large amount of zeaxanthin in group B might be that, although the chickens were consuming just half of the supplement level given to group C ([see figure 2](#)), they consumed more food than group C. We know this because all food was weighed before it was put in the troughs and leftover food was weighed again at the end of feeding. A second possible reason for the dramatic results seen in group B is that its baseline was lower than that of either group A or C ([see figure 3](#)). A third explanation could be the inherent idiosyncratic animal issues in group C. One chicken stopped laying eggs half way through the study, making it necessary to use one of the reserve chicken's eggs instead. While this new chicken had been eating the same diet as the other chickens in group C, individual variation in animal physiology may have compromised our results, minimizing the effects of L/Z supplement on group C.

When the lutein and zeaxanthin concentrations were combined, more significant changes were seen. Again, concentrations in group B increased more than group C. We also observed that L/Z concentrations in group A decreased.

## **Orange Yolk: Is it coming to a market near you?**

The color of the yolks, which failed to change as dramatically as expected with the addition of L/Z supplements, may have had to do with a high baseline color rating, which began at 8/9 and increased to only about 9/10 ([see figure 4](#)). These results agree with previous research conducted showing that the concentration of L/Z in the yolk increases with consumption of a supplement, but the color appearance plateaus (Nys, 2000). However, if baseline color wasn't so high to begin with, a stronger correlation might have been observed with supplement level, resulting L/Z yolk concentration, and color appearance.

Although the color did not increase significantly, there was a correlation between the color of the yolk and its L/Z concentration as can be seen in [figure 5](#), a fact that researchers could potentially use to predict the amount of L/Z in an egg simply by observing the yolk's color. However, the fact that the color changes very little as L/Z concentrations increase is commercially beneficial because if a darker color yolk was produced, it might not be appealing to the average egg consumer (Nys, 2000). Another aspect of this study that contradicted my expectations was a drop in L/Z concentration and color rating in group A. To explain this, we examined the feed the chickens consumed prior to the study. All animals consumed the control diet of Blue Seal™ feed for two weeks before the study began. However, the starter feed the chickens ate before the study was a special formulation that, upon further inspection, turned out to contain xanthophylls, a nutrient that the experiment feed did not contain. This change in diet—from one that supplied xanthophylls to one that didn't—resulted in a decline in L/Z concentrations in the control group. This also explains the less robust changes seen in group B and group C. By starting the chickens on the baseline diet earlier we may have seen more dramatic color changes than those that occurred. In future studies, the animals should be stabilized on the same control diet for a longer period of time.

## **In an Eggshell**

In conclusion, my results confirmed that egg yolk is an excellent source of L/Z and that the concentration of L/Z can be modified by regulating an animal's diet. Supplementing a chicken's diet with commercial xanthophylls, such as Oroglo™ or green leafy vegetables high in L/Z, will increase the egg yolk's L/Z concentration without negatively altering its color. This means that while most commercial eggs are not as rich in health-promoting xanthophylls as was demonstrated here, the L/Z concentration could easily be enhanced through dietary manipulation while maintaining egg yolk color that is acceptable to consumers.

I hope that this study encourages more people to eat eggs (including the yolk) because research clearly shows that they are filled with quality nutrients that can benefit our health. I really enjoyed doing this project not only because I learned a great deal about this topic and how to properly conduct research, but it was a great way to spend the summer. I encourage more students to embrace the research opportunities available to them because the experience for me proved quite beneficial.

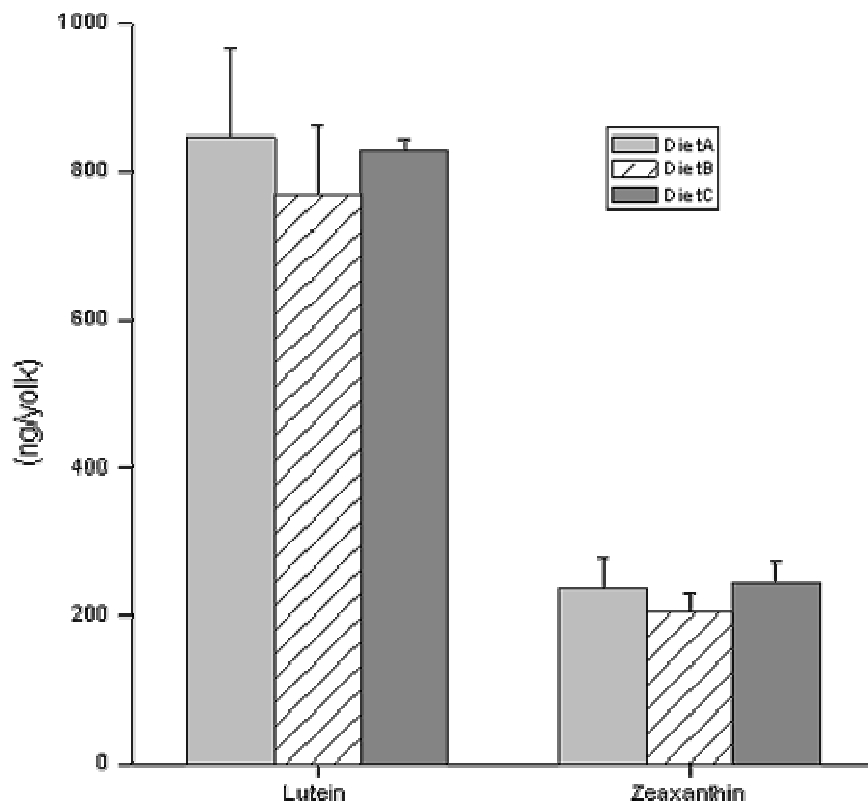
*This project could not have been done alone. I would like to thank Dr. Taylor for providing the chickens and their housing; Adam Wenzel for helping with the statistics and thinking about the project in graduate terms; Sue Jalbert for teaching me lab techniques; and Naomi Shevenell for helping to feed and visit the chickens every day. Also, a big thank you to Dr. Celentano for inspiring me to undertake the project, overseeing the experiment, and reviewing the accuracy of this research article.*

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## Appendix

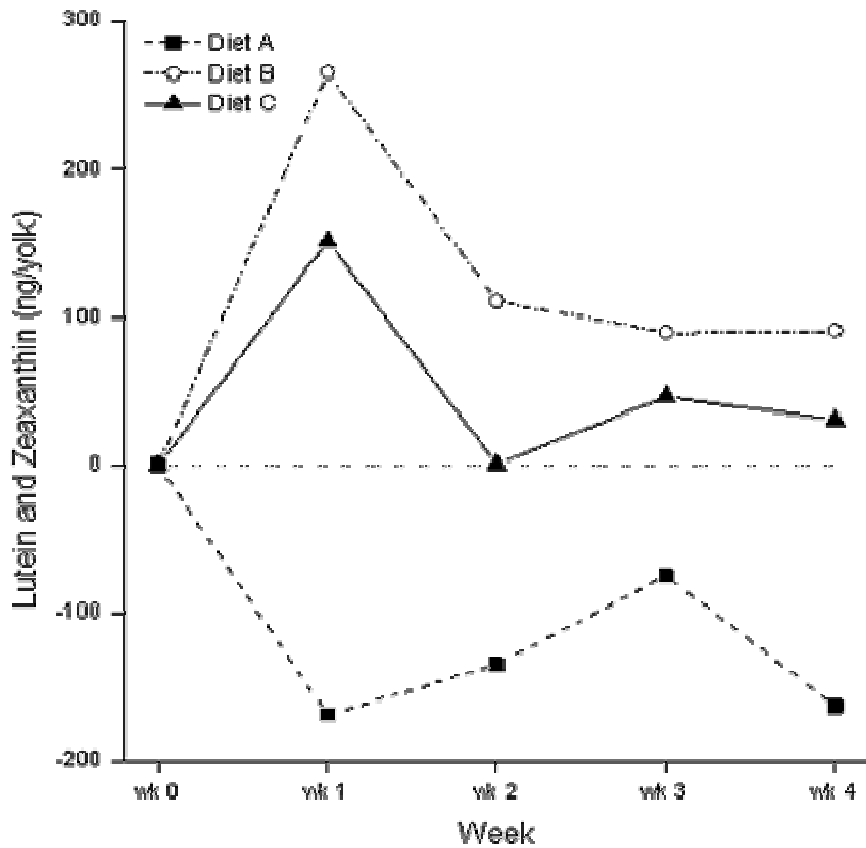
Figure 1: Comparing Baseline Measurements by Diet



**Figure 1:** Group B had a lower baseline concentration of lutein and zeaxanthin than Group A or C. This graph also shows the difference in concentration between lutein and zeaxanthin. Lutein is found in much higher concentrations than zeaxanthin, but they are usually found together.

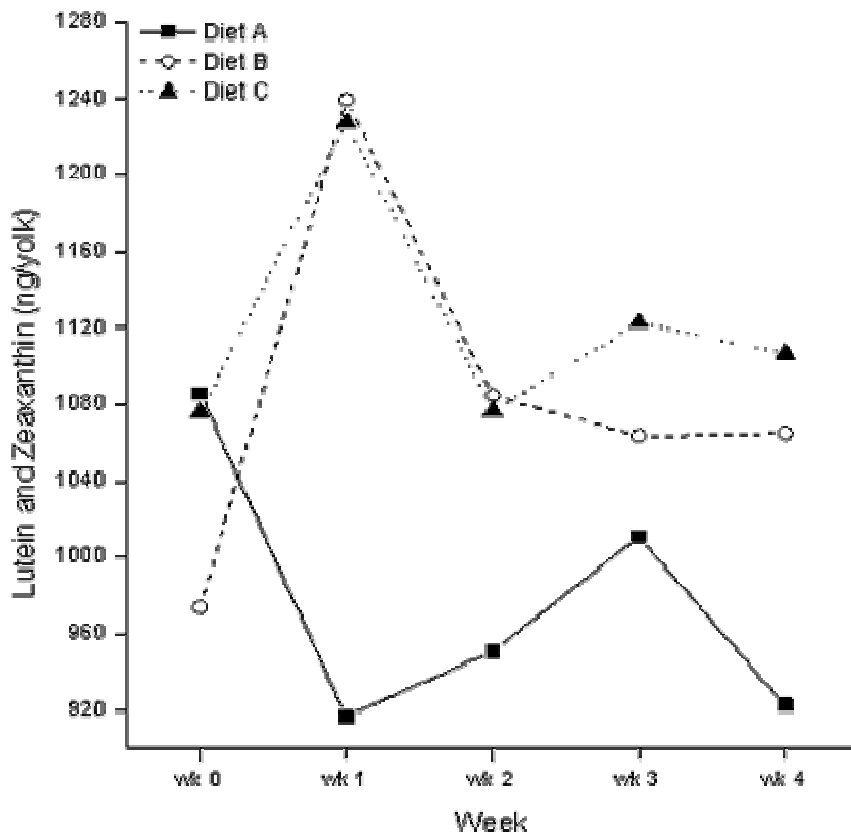


Figure 2: Lutein and Zeaxanthin Change from Baseline



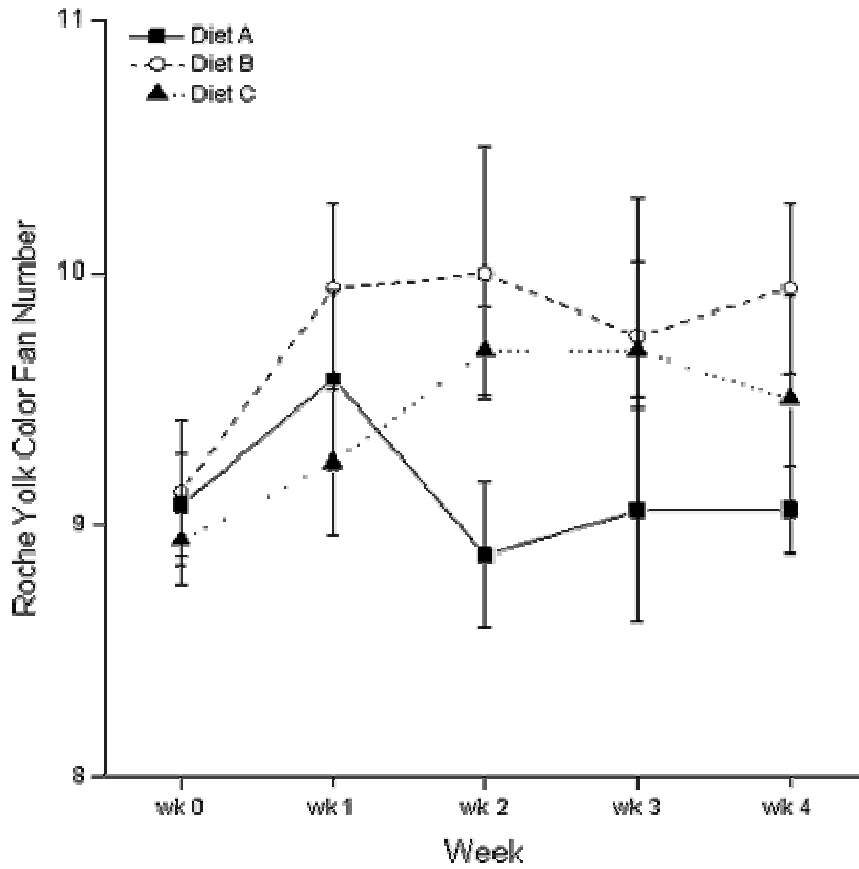
**Figure 2:** This graph shows how L/Z concentrations changed from the baseline measurement. There was a distinct decrease in L/Z concentration in group A, while group B shows a larger increase than group C. However, both Group B and C show a similar pattern in change from the baseline.

Figure 3: Lutein and Zeaxanthin Over Time by Diet



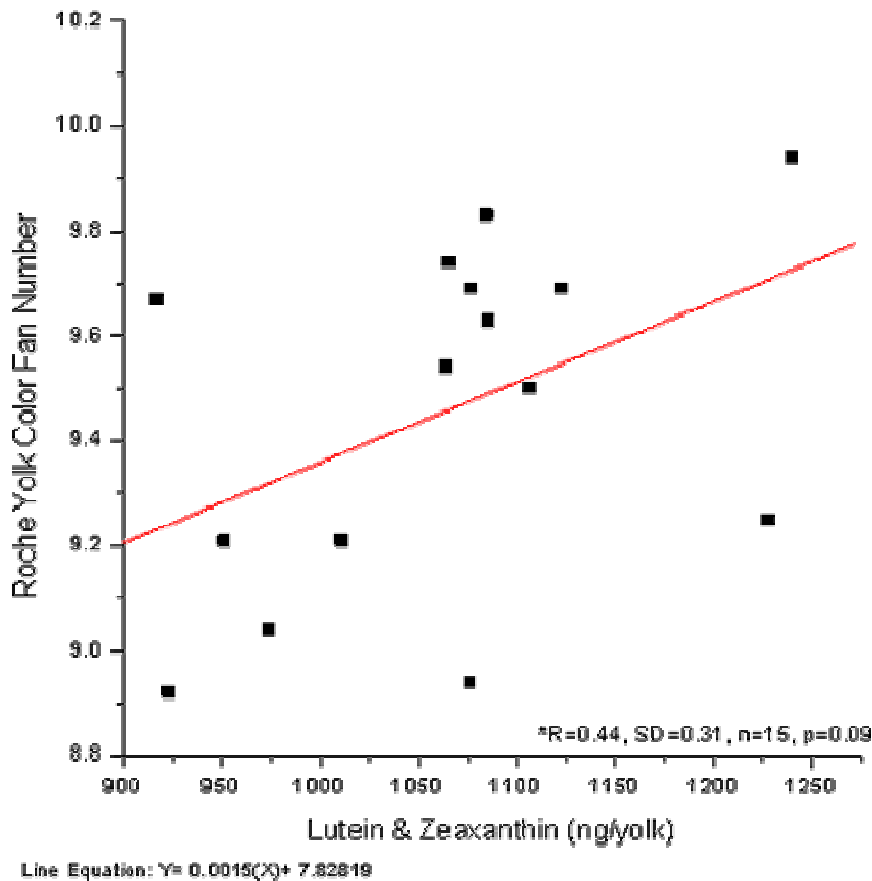
**Figure 3:** This graph is different from figure 2 in that it shows L/Z concentration changes over time from the actual baseline for each group. As can be seen here, group B had a much lower baseline than either group A or C. Again, group A shows a drop in concentration, while B and C show a similar pattern of change. Because this graph shows each individual baseline, you can see that group C had higher L/Z concentrations than group B during the last two weeks of the study.

Figure 4: Yolk Color Over Time by Diet



**Figure 4:** The yolks started out with similar colors across the treatment groups. As time went by, the group A yolks tended to have lower color ratings. Group B ended up with the highest color ratings, while group C came between the two other groups. This data is similar to what was seen with L/Z concentration in the respective treatment groups.

Figure 5: Lutein and Zeaxanthin Content to Roche Yolk Color Fan Number



**Figure 5:** The graph shows the correlation between L/Z content and the color rating of yolks. There was a fairly strong relationship observed between the two variables, showing that as L/Z concentration increased the color rating increased, but a few outlying numbers were present. With more data, this correlation could be more significant.

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### Author Bio

Born and raised in North Granby, Connecticut, **Gwen Gardner Stewart** grew up observing the growth and development of chickens on her parents' poultry farm for nearly ten years. After three years of study in nutritional science at the University of New Hampshire, Gwen's experience with poultry naturally transitioned into a research project funded by a Summer Undergraduate Research Fellowship (SURF). Upon graduation, Gwen plans to complete an internship in the field of nutrition and become a registered dietician. Although she had not originally considered a career in nutritional research, this project has certainly given her a new perspective on her options for the future. She looks forward to exploring those possibilities more through her internship.

### Mentor Bio

*A member of the University of New Hampshire's faculty since 1986, **Joanne Curran-Celentano** is a professor of nutritional sciences. She specializes in human nutrition with a focus on phytonutrients, an interest since her undergraduate years at Rutgers University. Over the course of her tenure at UNH, Dr. Curran-Celentano has been a mentor for ten students involved in research at the University, a job she finds very rewarding both for herself and for her students. "Being involved with research has provided [Gwen] the tools to understand first hand how data published in journals is derived and has given her a deep appreciation for the rigor necessary for drawing conclusions from data," she said. Dr. Curran-Celentano has a special interest in lutein bioavailability and its apparent link to human eye health and, in particular, with a decreased risk of Age-related Macular Degeneration (AMD).*