NATURAL XANTHOPHYLLS IN THE EGG

WHEN GOOD COLOR IS NOT ENOUGH

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BACKGROUND

It is a common practice to color egg yolks by adding pigments in the diet of layers. In fact, this phenomenon in nature is as old as the very animal kingdom.

CAROTENOIDs, AND MORE SPECIFICALLY XANTHOPHYLLS (CAROTENOIDs CONTAINING OXYGEN ATOMs WITHIN THEIR MOLECULEs) GIVE THE EGG YOLK ITS NATURAL COLOR.
Carotenoids, and more specifically xanthophylls (carotenoids containing oxygen atoms within their molecules) give the egg yolk its natural color.

Carotenoids also lend feathers their red, orange and yellow hues in many bird species, which has been identified as a sign of health and reproductive eligibility.

In traditional and self-feed farming systems, xanthophylls, primarily lutein and zeaxanthin, are present in dietary components such as corn, wild flowers, green vegetables, fruit, insects, etc.

The main xanthophylls are lutein and zeaxanthin, which are present in corn, wild flowers, green vegetables, fruit, and insects, among others.

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Modern poultry production has brought about continuous improvements in output levels and feed conversion, as a result of genetic, management and health enhancements.

However, this means that for every egg produced, layers take up less carotenoid from raw materials. This makes it necessary to balance out the levels supplied in the diet by additional supplementation.

In addition, direct supplementation of carotenoids has enabled unrestricted use of raw materials lacking significant levels of xanthophylls. The use of such external sources also guarantees lower variability of egg yolk pigmentation levels among animals and throughout their productive lives.

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Consumers associate intense yolk color with high egg quality and freshness. This perception is based on the popular knowledge that diseased hens absorb carotenoids poorly from the diet and lay eggs with pale tones.

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Egg yolk color can be assessed subjectively by means of color scales, such as the Roche Yolk Color Fan (RYCF), which comprises 15 categories, with a score of 15 indicating maximum pigmentation.

A more technical approach is to use reflectance colorimetry and apply a three-dimensional scale based on the CIE system (defining lightness or L*, yellowness or b*, and redness or a*).

Intense orange colors make eggs more desirable for consumption to a vast majority of consumers; however, there are considerable differences in preference, which are strongly linked to historical and cultural factors. Indeed, in the European Union, consumers in Spain, Italy, Germany and France generally prefer deeply colored eggs (RYCF 12-13).

Also, intensely colored eggs are highly demanded by the food industry for products such as pasta, pastries, sauces, etc. Good yolk pigmentation requires a base of so-called “yellow xanthophylls” (lutein; beta-apo-8’-carotenoic acid ethyl ester or apo-ester) and “red xanthophylls” (capsanthin; canthaxanthin), which can be used to attain a wide range of orange hues according to consumer preferences in each market.

### Yellow xanthophylls
- Lutein
- Acid ethyl ester
- Beta-apo-8’-carotenoic acid ethyl ester or apo-ester

### Red xanthophylls
- Capsanthin
- Canthaxanthin
NATURAL PIGMENTS

Key natural pigments include marigold (Tagetes erecta) flower extract, which is rich in lutein and zeaxanthin, and red pepper or paprika (Capsicum annuum) extract, which is rich in capsanthin.

HOW TO OBTAIN AND PROCESS THE MARIGOLD FLOWER EXTRACT?

As with any vegetal extract, organic solvents are used for extraction and the vegetable matrix is treated to optimize separation and collection of xanthophylls and other fat-soluble components.

This ‘raw’ extract primarily contains esterified xanthophylls, i.e. xanthophylls linked to fatty acids. Esterified xanthophylls need to be released from the fatty acids for absorption in the gut, but intestinal lipases in birds hydrolyze these esters only partially.

For this reason, the vegetable extract is subjected to a saponification process whereby xanthophyll esters are transformed into free xanthophylls, which are much more bioavailable.

In addition, xanthophylls are isomerized (the molecule is rotated at a specific double-bond), as the trans isomer of xanthophylls is more stable and has more redness than the cis isomer (Hamilton et al., 1990).

The saponified and isomerized extract is then protected and stabilized using relevant antioxidants included in a macrogranular solid matrix or diluted in water, for subsequent addition to bird feed as a solid or liquid.

SYNTHETIC OR NATURAL IDENTICAL PIGMENTS

Canthaxanthin and beta-apo-8’-carotenoic acid ethyl ester (apo-ester) are the most relevant synthetic xanthophylls in poultry. They are produced from acetone and β ionone (Isler et al., 1967). These xanthophylls are found in free forms and are included in matrices of saturated fatty acids and antioxidants for protection and stabilization.

Sometimes these carotenoids produced entirely by chemical synthesis are linked to the term ‘natural’ when described as ‘natural-like’ or ‘natural identical’, but this should not be misleading.

XANTHOPHYLLS FROM MARIGOLD EXTRACT ARE SAPONIFIED TO BECOME MORE BIOAVAILABLE

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Legal framework of the use of pigments

- The addition of natural and synthetic carotenoids in layer feeds in the European Union is currently limited to 80 ppm (mg/kg). The only exception is canthaxanthin, which is limited to 8 ppm in layers. This restriction aims to prevent the unwanted effects of excessive exposure in humans, as excessive levels of canthaxanthin can lead to the development of precipitated crystals in the retina (Arden et al., 1991; Baker, 2001).
- The use of carotenoids derived from vegetable extracts has been criticized by some in favor of synthetic carotenoids, simply because vegetable crops are exposed to potential contamination by environmental pollutants (dioxins, heavy metals, pesticides, etc.).

However, this could be applicable to any extract or product of vegetable origin, and therefore should never be a major reason to justify their replacement for synthetic products.

PIGMENTATION EFFICACY

Carotenoids are absorbed in the small intestine along with other lipid components and are transported by lipoproteins in the blood to the target tissues where they deposit — the egg yolk in layers.

THE PIGMENTING POTENTIAL OF EACH CAROTENOID IS DETERMINED BY THE EFFICACY OF INTESTINAL ABSORPTION AND ITS SPECIFIC AFFINITY FOR DEPOSITION IN THE YOLK
Despite the widespread idea that the pigmentation efficacy ratio between synthetic yellow (beta-apo-8’-carotenoic acid ester) and natural yellow (marigold extract lutein) is 1:3, two important factors explain why this ratio is actually closer to 1:1.5 in products such as the CAPSANTAL EBS range.

**Saponified vegetable extract**
- Marigold (Tagetes erecta) extract is used in many published studies with no subsequent saponification/isomerization process as described above.
- This limits the intestinal absorption of the extract. In fact, it has been shown that free lutein can double its absorption and deposition in the egg (Hamilton et al, 1990; Galobart et al, 2004).
- Furthermore, the characteristics of the excipients and antioxidants used to stabilize and homogenize the saponified extract help improve their pigmenting efficacy versus other raw extracts.

**Combined yellow and red pigments**
- One of the limitations of many published studies is the consideration of unreal situations regarding the use of xanthophylls in poultry. In some studies, synthetic yellow pigments are compared to natural yellow pigments using increasing doses up to levels much higher than those used customarily, and no natural (capsanthin) or synthetic (canthaxanthin) red xanthophylls are added. This is also the case when natural and synthetic red carotenoids are compared without using a yellow base.
- Interestingly, in order to obtain pigmentation above 9-10 on the RYCF, with a bright yellow-orange color that pleases the eye, yellow and red pigments should be combined, unless the raw material (basically, corn) already contains the necessary yellow xanthophylls. Therefore, it is essential to associate the efficacy of a specific concentration of yellow xanthophylls with the presence of a specific concentration of red xanthophylls, and vice versa.
- This is not always easy, as it entails designing experimental tests with more treatment and more animals, and greater complexity in the careful interpretation of results.
- Over the years, we have conducted numerous studies on our experimental farm using our marigold flower extract (CAPSANTAL EBS). These studies have confirmed the aforementioned 1.5:1 ratio versus synthetic yellow xanthophyll apo-esters.

**Recent trials**
- As an example, below are our most recent results in 23-week-old Hy-Line Brown hens treated for 6 weeks at the IRTA facilities.
- Target pigmentation was defined as 12 on the RYCF. This can be considered to be the standard for Spain and other countries with a preference for deeply colored table eggs.
- A standard dose of 2.5 ppm canthaxanthin was added to the diet as a supplement for red xanthophylls.
- At the feed addition levels evaluated, no differences were seen between the synthetic apo-ester source (2.5 ppm) and marigold extract CAPSANTAL EBS 30 NT (3.75 ppm), as assessed by colorimetry or using the RYCF (Figure 1).

**In addition,** we have conducted studies to compare the ratio of natural red pigments (capsanthin) from saponified red pepper extracts (CAPSANTAL FS) to commercial sources of canthaxanthin. For this comparison, a base of yellow carotenoid from saponified marigold extract was also included. In this case, deposition efficacy of the saponified paprika extract was 2-2.5:1 in favor of canthaxanthin (ITPSA, 2005; EFSA, 2006).

**NUTRACEUTICAL SOURCE OF LUTEIN**

When considering not just the coloring effect of xanthophylls but also their physiological benefits such as antioxidant activity, prevention of certain cancers, effects on cardiovascular disease, immune system stimulation and anti-inflammatory properties, as well as their effect on ocular physiology and tissue ageing, the importance gained by these pigments over the last few years becomes apparent, not only for their organoleptic implications but also for their nutritional and nutraceutical qualities.

In fact, these carotenoids in the yolk are originally intended in nature to offer antioxidant and immune protection to the hen and the developing embryo (Karadas et al, 2005).
Lutein benefits

In humans, one of the key benefits of lutein in the diet is its accumulation in the retinal macula, which increases macular pigment optical density. This effect is exclusive to lutein and zeaxanthin.

It has been suggested that lutein and zeaxanthin confer protection against age-related macular degeneration and cataracts, which are the main causes of blindness in developed countries (Olmedilla et al, 2003). Such protection is believed to be linked to the role of lutein in the retina as a filter of ultraviolet light and its power as a natural antioxidant (Snodderly, 1995).

Additionally, a number of interesting studies have been published over the last few months in which macular levels of lutein are associated with significant levels of lutein in brain tissues; this has been associated with an improvement in cognitive function and intellectual performance (Johson, 2014). Furthermore, lutein deficits have been seen in the brains of premature newborns (Vishwanathan et al, 2014).

In any case, lutein and zeaxanthin make up the bulk of the carotenoids taken up by hens in their natural diet and account for more than 85% of total carotenoids in commercial eggs (Goodrow et al, 2006; Jang et al, 2014).

In addition, lutein and zeaxanthin are the only carotenoids found consistently in organic eggs, with levels twice and three times as high as those found in conventionally produced eggs. In this regard, xanthophyll profiles can be good markers to differentiate eggs from different types of production. Just as xanthophylls are used to obtain desirable yolk colors, marigold extracts can be added to increase lutein deposition and obtain enriched eggs with a nutraceutical profile.

THE BENEFITS OF LUTEIN EXPLAINS THE CURRENT INTEREST IN THE BIOLOGICAL VALUE OF THE EGG YOLK

This accounts for the current interest for appreciating the contents of lutein in eggs, especially considering that carotenoids from egg yolks have greater biological value, i.e. greater bioavailability and presence in tissues following their absorption (Chung et al, 2004).

In fact, it has been suggested that 1 mg of egg lutein has as much bioavailability as 5 mg of lutein from synthetic dietary vegetable supplements (Kelly et al, 2014)

The increase in bioavailability seen in eggs is due to the highly digestible lipid matrix and the absorption of the yolk.

Several studies show that commercial egg yolks contain 0.2 mg to 0.5 mg lutein, with a concentration of approximately 3.5 ppm to 9 ppm. Similar values were seen for its isomer zeaxanthin (Perry et al, 2009; Rasmussen et al, 2012).

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Taking as a reference the maximum level allowed in the European Union for lutein in the feed of layers (80 ppm), levels above 25 ppm were obtained in the egg. Assuming that the yolk makes up 35% of the egg, yolk levels above 40 ppm are obtained. This equals 1.5 mg lutein in each egg. These levels of lutein are three to seven times higher than those found in commercial eggs (0.2-0.5 mg/egg).

Recent trials

For these purposes, a test was conducted on ITPSA’s experimental farm (province of Tarragona, Spain) using our marigold extract rich in unesterified lutein (CAPSANTAL EBS) in 56-week-old Isa-Brown layers in pens; egg enrichment was evaluated. Wheat-barley and soy feeds were used.

The results obtained confirmed a direct relationship between diet lutein and lutein levels in the egg yolk. In addition, the efficacy of deposition decreases with increasing levels in the diet of layers, going from 25% to less than 10% (Figure 2 and Figure 3).

Such supply of free lutein from the egg is not negligible considering that the daily recommended intake of lutein for an adult person has been estimated to be 10-20 mg per day and that the mean daily intake of lutein for an adult is close to 1 mg per day (Andorssum and Bone, 2001; Leeson and Caster, 2004).
LOOKING AT THE FUTURE

As we have seen, it is feasible to significantly enrich the xanthophyll contents of commercial eggs, especially in lutein-zeaxanthin of high biological value.

Because of such higher bioavailability compared to vegetable sources, several studies and ongoing research projects sponsored with European funds are focusing on the use of lutein from eggs for addition to other foods.

For instance, several research groups in the Netherlands are developing nutritional supplements in the form of dairy beverages incorporating enriched egg yolk, which ensures the intake of about 1 mg lutein and 0.35 mg zeaxanthin (Kelly et al, 2014). This supplement increased blood levels of lutein and zeaxanthin by more than 75%. In another study, it was shown that blood lutein levels increased by 83% in individuals who took a buttermilk drink enriched with the equivalent of 1.5 egg yolks daily for one year (Van der Made et al., 2014). This line of research will continue in the future.

PIGMENTATION SHOULD BE BASED ON BOTH RED AND YELLOW XANTHOPHYLLS TO ADJUST TO REAL CONDITIONS OF COMBINED USE IN THE POULTRY INDUSTRY

XANTHOPHYLLS FROM VEGETABLE EXTRACTS ADD NUTRACEUTICAL VALUE TO THE EGG BY INCREASING THE LEVELS OF LUTEIN IN COMMERCIAL EGGS BY UP TO 7 TIMES

EGG LUTEIN IS RECEIVING RENEWED INTEREST FOR ITS USE IN NUTRITIONAL SUPPLEMENTS GIVEN ITS BENEFITS AS A FUNCTIONAL FOOD

References: To be provided upon request.