# The beauty of pigments is more than skin deep

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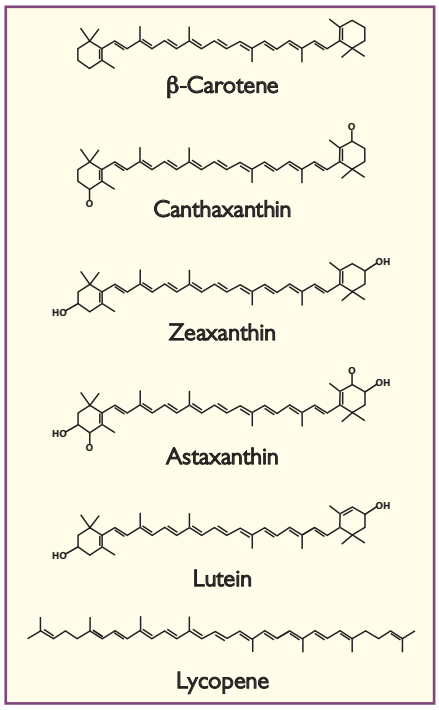
Yellow xanthophyll pigments are among the cornerstone additives in poultry diets for their benefits on skin and egg characteristics. However, continued research is giving insight to impact on antioxidant and immune systems in the animal which may bring animal performance to another level.

Carotenoids are the primary source of pigmentation in poultry, fish and shrimp. Pigmentation is an important factor in consumer acceptance and perceived quality of these food sources. Normally, pigmentation characteristics can be obtained through carotenoid content in raw materials in commercial feeds. However, their variable content and stability have led the industry to use more standardised sources of carotenoids from the marigold flower (Tagetes spp.) and red pepper (Capsicum sp.). Poultry use carotenoids for pigmentation, however these substances are also involved in growth metabolism and fertility.

***Fig. 1. Structures of most common carotenoids used in animal feed diets.***




Progress in laboratory analytical methodology have helped characterise the active ingredients by structure and function, and yielded more concentrated and stable formulations. Carotenoids belong to the category of tetraterpenoids (they contain 40 carbon atoms). Structurally they contain only carbon and hydrogen and are in the form of a polyene chain which is sometimes terminated by rings. Carotenoids that are unoxygenated (oxygen-free) such as alpha-carotene, β-carotene and lycopene are known as carotenes. Carotenoids containing oxygen, such as lutein and zeaxanthin, are known as xanthophylls.

The latter are particularly sensitive and unstable under normal storage conditions or feed production. The stability of xanthophylls preparations have been improved by a select few leading companies in the area using combinations of extraction methods, stabilising formulations and encapsulation technologies (as shown in the photograph in the top right hand corner).

These advances have produced formulations with significantly higher stability quotients for longer shelf lives and higher pelleting and expanding recoveries.

## Determining bioavailability

Differential absorption studies of carotenoids have been the focus of research for nearly three decades. The bioavailability of carotenoids is extremely variable,being determined by many dietary and physiological factors known as SLAMENGHI:

- Carotenoid species (S).
- Molecular linkage (L).
- Amount consumed (A).
- Carotenoid matrix (M).
- Effectors of absorption and bioconversion (E).
- Nutrient status of the animal (N).
- Genetic factors (G).
- Host related factors (H).
- Interactions (I).

As seen from the SLAMENGHI list, the most determining factor remains the structure. The absorption of dietary carotenoids

involve several steps starting with the mechanical and enzymatic disruption of the feed matrix, release of the carotenoids, followed by their incorporation into loipid droplets of the gastric emulsions.

The carotenoids are then transferred from the lipid droplets to mixed micelles produced by the action of bile salts, biliary phospholipids, dietary lipids, and their hydrolysis products.

After the solubilisation in mixed micelles, carotenoids are absorbed by the intestinal cells, packed into chylomicrons and secreted to the lymphatic system.

It has since been accepted that there are regulatory mechanisms for carotenoids across the small intestine epithelium, where carotenoids are essentially 'ready' for absorption across the small intestinal epithelium (enterocytes).

In contrast, steps in micellation tend to hint to differences between carotenoids, and especially carotenes (β-carotene, lycopene) versus xanthophylls (lutein, zeaxanthin). Mixed micelles are formed in the duodenum by the detergent action of biles salts and lipid emulsion droplets.

The structural nature of carotenes such as β-carotene is highly dependent on fat level in the diet (higher fat affording more micellarisation of carotenes), while xanthophylls are less dependent (to the point of being independent of fat level). The difference appears to be in the intermediate step of lipid droplet formation (see Fig. 2).

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Lutein has been a molecule in focus for its use in prevention of macular degeneration and other end-organ disease. This is in consequence of its high bioavailability and its immune and antioxidant mediating actions.

## Potential of dietary lutein

Several studies by Kurt Klasing and his colleagues at the University of Davis, California in the USA have shown the potential of dietary lutein to modulate immunity, particular in leukocytes and macrophages.

The action appears to be through nitric oxide synthase, thereby modifying the production of nitrites.

Lutein's antioxidant actions remain to be defined, but may be both primary and secondary in nature.

With approximately 1-2% of metabolised oxygen being converted to a reactive oxygen species, the need for efficient biochemical molecules and systems in both cells and extracellular fluid their removal are needed.

Antioxidant systems include β-carotene and vitamin E are those already used to maintain the integrity of phospholipids against oxidative damage and peroxidation, but others are sought. Indeed, increased generation of free radicals may overwhelm antioxidant defence mechanisms and compromise cellular function.

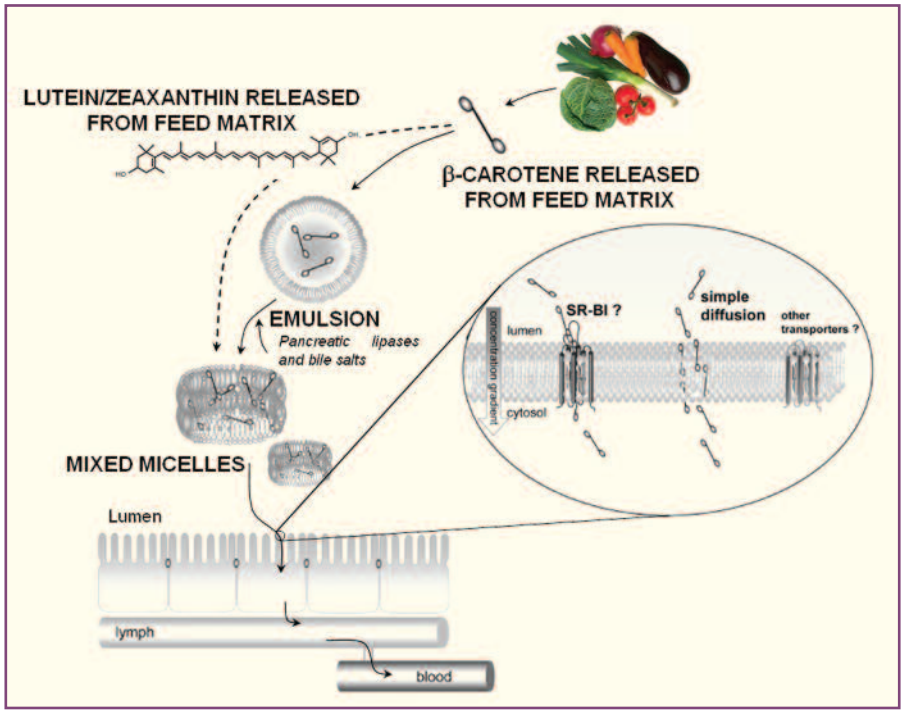


***Fig. 2. Comparison of proposed digestion and absorption pathway of lutein versus β-carotene. Note that lutein 'bypasses' the emulsion step taken by carotenes.***

Production of free radicals could represent a source of infertility because ovarian steroidogenic tissue, spermatozoa and preimplantation embryos are sensitive to free radical damage.

It is believed that oxygen radicals also interfere with female germ cell development and maturation which occurs in the context of an ovarian follicle, which contains a germ cell (oocyte or egg) and somatic granulosa and theca cells.

Intercommunication between oocytes and granulosa cells is necessary for proper oocyte and follicular development.

Maintaining the functional architecture between oocytes and granulosa cells promotes production of a fertile oocyte. Several micronutrients such as ascorbic acid or β-carotene (a pro-vitamin A source) have been shown to improve several characteristics of oocyte maturation. Achieving full in vitro growth of oocytes of both domestic animals and humans remains a major challenge.

Along this objective, a preliminary study was commissioned to determine the effects of lutein on the in vitro development of primary follicules. Isolated primary follicules (mean diameter 60.4 ±0.8μ) were cultured in serum free medium on fibronectin coated wells for 42 days in the absence (-LUT) or presence (+LUT) of 2 uM of filtered oleoresin from Tagetes spp (80% lutein; 5% zeaxanthin).

All treatments were started on day three of culture. At day 14, oocytes were graded by the absence of granulosa cells.

The growth of granulosa cells around the oocytes were an indication of follicle-like units (reorganised follicules; secondary follicles).

The diameter of the follicle units were also recorded. Treatments were performed in replicates of three. The results of this study are shown in Table 1.

Although preliminary, the results of this study support the observation that the supplementation of lutein (from Tagetes spp.) enhanced the development of primary follicles to reach the secondary follicle stage.

Further studies are need to determine the nature of its action, but lutein may exert its effect through its antioxidant function.

## Conclusions

Research is currently ongoing into the effects of the active ingredients of carotenoids such as lutein. While once used for its benefits on the physical aspects of egg and skin, lutein may have even more added value as an impact ingredient on development and fertility. ■

***Table 1. Effects on percentage and size of follicle units of oocytes in vitro after 14 days in culture.***

| Parameter | Negative control | Negative control + Tagetes spp | Difference (%) |
|---|---|---|---|
| Follicle units | 14/36 | 23/36 | +30 |
| Diameter size (μ) | 44.1±1.2 | 65.3±2.6 | +32 |