

*Review Article***Use of carotenoids in feed mixtures for poultry: a review**Milan Marounek<sup>1</sup>, Akhir Pebriansyah<sup>2</sup><sup>1</sup>*Institute of Animal Science, Department of Nutrition Physiology and Animal Products Quality, Prague 22, Czech Republic*<sup>2</sup>*Czech University of Life Sciences, Faculty of Tropical AgriSciences, Department of Animal Science and Food Processing, Prague 6, Czech Republic***Correspondence to:****Milan Marounek**, Institute of Animal Science, Přátelství 815, Prague 22, 104 00, Czech Republic

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**Abstract**

Carotenoids are present in ingredients of feed for poultry. Their content can be further increased by supplementation of feed mixtures with synthetic or natural carotenoids. The present paper recapitulates knowledge on the use of carotenoids in broiler chickens and laying hens, and deals with synthetic or natural carotenoids derived from plants. The review describes effects of carotenoids on productive performance, oxidative stability of poultry products, colouring of yolks and accumulation of carotenoids in yolks. There are several alternatives to synthetic carotenoids which are usable in poultry diets and satisfy the interest of consumers in poultry products free of undesirable side effects.

**Keywords:** carotenoids; poultry; chickens; laying hens; eggs.**INTRODUCTION**

Carotenoids are organic pigments synthesized from isoprene units in plants and algae. Carotenoids represent one of the most widespread groups of natural pigments. They are synthesized *de novo* by plants, but they are present in animals, where they accumulate either unchanged from the diet, or metabolically modified. There are over 1100 known carotenoids (Yabuzaki, 2017). The characteristic carotenoid pattern in birds is the accumulation of xanthophylls (i.e. carotenoids containing at least one oxygen atom in the molecule), and the almost complete exclusion of carotenes (oxygen-free carotenoids). Carotenes such as  $\beta$ -carotene,  $\alpha$ -carotene and  $\varepsilon$ -carotene represent the subclass of unsaturated hydrocarbons (Goodwin, 1986). Plant carotenoids are the primary dietary source of provitamins A, with  $\beta$ -carotene as the best-known example. Others include  $\alpha$ -carotene and cryptoxanthin. Carotenoids with high vitamin A activity have low pigmentation properties (Hencken 1992). The essential structural requirement in the provitamin A molecule is one unsubstituted ring attached to an intact conjugated polyene structure, thus  $\beta$ -carotene with two essential structural units is the most potent provitamin A. The main site of the conversion of  $\beta$ -carotene to vitamin A is the intestinal mucosa and two enzymes

are involved: oxygenase which splits the molecule in the centre to yield retinal (vitamin A aldehyde) and retinal reductase that converts retinal into retinol (Goodwin, 1986). Retinol and metabolites such as retinoic acid play major roles in vision, immune and brain functions, tissue remodelling and metabolism (Brossaud et al., 2017).

It has been shown that the absorption of lipophilic vitamins requires to be combined with bile acids and lipid to form micelles (Preveraud et al. 2015, Silva and Furlanetto, 2018). Presumably, also intestinal absorption of carotenoids in poultry may be enhanced by the presence of fat in feed mixtures. This assumption is worth of future experiments.

**CAROTENOIDS IN POULTRY INDUSTRY**

In the poultry industry carotenoids have been used as means of pigmentation of eggs and skin. The EU Register of Feed Additives (2018) lists xanthophylls, i.e. which can be added to animal feed. List of the authorised additives includes capsanthin,  $\beta$ -apo-8'-carotenoic acid ethyl ester, lutein, canthaxanthin, zeaxanthin, and citranaxanthin (Table 1.).

Maximum content of canthaxanthin in the feed of laying hens is 8 mg per kg, in poultry other than laying hens is 25 mg per kg. Maximum content of

**Table 1.** List of the authorised additives in feedingstuffs of poultry\*

Additive	(EC no)	Species or category of poultry
Capsanthin	(E 160c)	Poultry
$\beta$ -apo-8'-carotenoic acid ethyl ester	(E 160f)	Poultry
Lutein	(E 161b)	Poultry
Zeaxanthin	(E 161h)	Poultry
Citraxanthin	(E 161i)	Laying hens
Canthaxanthin	(E 161g)	Chickens for fattening Laying poultry

\*European Union Register of Feed Additives pursuant to Regulation (EC) No. 1831/2003. Annex I: List of additives. Edition 2/2018.

other xanthophylls which are produced synthetically is 80 mg per kg (Breithaupt, 2007). Traditional producers of xanthophylls, are companies DSM Nutritional Products and Badische Anilin & Soda Fabrik (BASF). Due to a lengthy synthesis lutein is not produced synthetically, but extracted from petals of marigold (*Tagetes erecta*). The supplementation of feed mixtures for poultry with carotenoids is suitable in all poultry categories, although reasons are partially different: the pigmentation of meat and skin in broiler chickens and the optimum yolk colour in laying hens. The supplementation of poultry diets with carotenoids increases the oxidative stability of poultry products. The carry-over of xanthophylls in the human food chain could also be advantageous for human health, especially because lutein and zeaxanthin are deposited in the macular region of the retina and reduce the risk of age-related macular degeneration (Landrum et al. 1999). Carotenes are isoprenoids, synthesized from isoprene units. Due to very low polarity, the absorption of carotenes is low, consequently also their pigmentation activity, as observed more distinctly in layers (Hammershøj et al. 2010).

#### Effect of carotenoids in broiler chickens

Ruiz et al. (1999) studied the effect of  $\beta$ -carotene and vitamin E on oxidative stability in leg meat of broilers fed different fats. Effect of  $\beta$ -carotene on oxidation depended on dietary fat and its concentration in feed. Beta-carotene at 15 mg/kg acted as antioxidant in fresh and cooked meat, in stored meat, however, worsened the oxidative stability in stored meat at 50 mg/kg. Probable reason of it was a decrease in vitamin E content and direct prooxidant effect. The antagonism of  $\beta$ -carotene and vitamin E was confirmed by Carreras et al. (2004). In the breast meat of broilers  $\beta$ -carotene in the diet tended to limit the vitamin E accumulation. The authors concluded that  $\beta$ -carotene can act as both an antioxidant and a pro-oxidant depending on the dose.

Lycopene is a tetraterpene assembled from eight isoprene units. Lycopene consists entirely of carbon and hydrogen, thus it is also a carotene. Lycopene is a key intermediate in the biosynthesis of carotenoids.

Ševčíková et al. (2008) observed that dietary supplement of 100 mg/kg lycopene increased the final live weight of Ross 308 broiler chickens. Dietary lycopene positively affected the lipid profile of plasma (the higher content of HDL cholesterol and lower content of LDL cholesterol).

Engelmaierová et al. (2011) tested the effect of lycopene (0 and 75 mg/kg) and vitamin E (0, 50 and 100 mg/kg) in the diet of chickens. Lycopene without vitamin E had no effect on performance. The synergism of both antioxidants, however, improved the growth performance and oxidative stability of meat in fresh leg meat and in meat that had been stored for 3 days. In addition, lycopene reduced the cholesterol content of leg meat.

#### Supplementation of feed mixtures for laying hens with synthetic carotenoids

In poultry farming, synthetic xanthophylls are used as feed supplements to obtain optimum colouring of the broiler skin and the egg yolk. Colour is an important characteristic and selection criterion for food choice by consumers. Northern countries of the EU prefer weakly coloured yolks, whereas Southern countries of Europe prefer more intensively coloured yolks. The intensity and the colour of the yolk can be controlled by the type and concentration of dietary carotenoids: canthaxanthin, which is preferred red, and  $\beta$ -apo-8'-carotenoic acid ethyl ester, which is preferred yellow. Both carotenoids are commercially available. Canthaxanthin is available as Carophyll<sup>®</sup>Red (DSM Nutritional Products, Basel, Switzerland), and  $\beta$ -apo-8'-carotenoic acid ethyl ester as Carophyll<sup>®</sup>Yellow, from the same company. Corresponding xanthophylls produced by BASF (Ludwigshafen, Germany) are Lucantin<sup>®</sup>Red and Lucantin<sup>®</sup>Yellow.

In humans, a part of the ingested canthaxanthin is absorbed (9 to 34%) and its elimination is characterized by a long half-life of about 5 days. The acute oral toxicity of canthaxanthin is very low. However, several animal studies indicate that daily oral administration of canthaxanthin was associated with crystalline deposits in the retina (EFSA 2010). Based on this finding, canthaxanthin can be added to

the feed of laying hens at a maximum of 8 mg/kg and  $\beta$ -apo-8'-carotenoic acid ethyl ester at 80 mg/kg.

In an experiment with Shaver Starcross laying hens at 42 weeks of age Halaj et al. (1999) reported that synthetic carotenoid Carophyll increased the laying intensity by 2.2% and the egg weight by 4.7%. No details of dosing of Carophyll were present. Contrary to this, Rosa et al. (2012) reported that the body weight, mortality and laying rate were not affected by the inclusion of Carophyll<sup>®</sup> Red in the diet of broiler breeders.

A meta-analysis on the effect of canthaxanthin on egg production in brown egg layers was published recently by Faruk et al. (2018). The data set contained 576 performance measurements from 34 trials and the age of layers ranged from 21 to 65 weeks. The canthaxanthin supplementation was in the range of 0 to 8 mg mg/kg of feed. A significant dose-dependent increases were found in egg yolk mass (+0.53% per mg of canthaxanthin), egg weight, and in feed intake. A numerical increase was found in egg production (+0.28%). The deposition of canthaxanthin in the egg yolk was 2.25 mg/kg per 1 mg/kg of canthaxanthin in the feed. Canthaxanthin is approved for use in EU, U.S.A. and Canada, but not in Australia and New Zealand.

#### **Supplementation of fed mixtures for laying hens with lutein and lycopene**

Lutein is oxygenated carotenoid, present in green leafy vegetables and yellow carrots. In plants it is present as fatty acid esters. Lutein and its isomer zeaxanthin accumulate in the retina. Both carotenoids decrease the risk of age-related macular development and the risk of cataract development. Lutein is isolated from the petals of marigold by extraction with organic solvents. In laying hens fed diets supplemented with lutein at 500 mg/kg the lutein content in eggs increased to 2.2 mg/60 g, as well as the Roche colour score of yolk. There was no increase in yolk lutein with diet supplements > 375 mg/kg (Leeson and Caston, 2004). In the subsequent experiment of Leeson et al. (2007) lutein was added to diets of laying hens at 125 and 250 mg/kg. Lutein significantly increased the lutein content from 0.10 to 1.60 mg/60 g egg, yolk colour and hepatic lutein concentration. There was no effect of lutein on the performance of layers, egg weight and eggshell deformation.

Golzar Adabi et al. (2010) observed that the plasma content of lutein was significantly increased in human adults who consumed one egg daily of hens fed diets supplemented with lutein at 500 and 750 mg/kg. Wenzel et al. (2006) concluded that the concentrations of carotenoids in eggs may be modest to other sources, such as spinach, however their bioavailability to the retina appears to be high.

Lycopene is the oxygen-free carotenoid of the carotene family, present in fruits and vegetables. Although lycopene is chemically a carotene, it has no provitamin A activity. Common dietary sources are tomatoes and tomato products. Lycopene is a strong antioxidant. Data of Olson et al. (2008) show that in hens fed diets containing lycopene at 65–650 mg/kg lycopene was well deposited in egg yolk. The optimal lycopene incorporation occurred at 420 mg/kg diet. Resulting yolk lycopene concentration was about 4 mg/kg.

#### **Supplementation of feed mixtures for poultry with natural carotenoids**

At the present time, consumers have become more concerned about the use of synthetic additives in foods and feeds, thus the interest in natural alternatives has increased. Synthetic carotenoids are not allowed in organic agriculture, also lutein is not allowed because organic solvents are used for the extraction of lutein from flowers of marigold.

Suitable alternatives to synthetic carotenoids are tomato powder, marigold extract, lucerne meal and algae. Tomato powder is a natural source of lycopene. Akdemir et al. (2012) added tomato powder at 5 or 10 g per kg of basal diet of laying hens in mid-lay. Tomato powder increased serum and yolk lycopene,  $\beta$ -carotene, lutein and vitamin A. The performance and egg production persistency were improved in treated groups.

The marigold flower meal and marigold flower extract represent rich sources of carotenoids, mainly lutein and zeaxanthin. In the experiment of Lokaewmanee et al. (2011) lutein at 40 mg/kg diet supplied as marigold flower extract increased the redness of yolks, but did not influence performance parameters. In the experiment of Skřivan et al. (2016) marigold flower extract was added to a maize-wheat-soyabean diet of hens at 150 to 950 mg/kg. The extract used in this study contained 21.3 g of lutein and 9.6 g of zeaxanthin per kg. The hen-day egg production and egg weight were significantly increased in treated groups. The content of lutein and zeaxanthin, yolk colour parameters and oxidative stability yolk lipids were increased. The most recommended dose of the marigold flower extract would be 550 mg per kg of diet. At this dosing egg yolks contained lutein and zeaxanthin at 30.3 and 18.9 mg/kg dry matter, respectively, which increased the basal lutein and zeaxanthin concentrations by 45.3% and 119.6%, respectively.

Algae are polyphyletic organisms, mostly aquatic. Algae attracted the attention of nutritionists due to their high content of carotenoids and unsaturated fatty acids. Some examples of the use of algae in diets of hens are presented hereinafter:

Herber-McNeill and Van Elswyk (1998) supplemented hen diets with marine algae. Feeding this product increased yolk redness as early as within one week feeding.

Fredriksson et al. (2006) observed that the addition of marine microalgae *Nannochloropsis oculata* to hen diets increased content of eicosapentaenoic and docosahexaenoic acid in egg lipids. Long chained fatty acids were almost exclusively present in the fraction of phospholipids.

Zahroojian et al. (2011) concluded that marine algae *Spirulina platensis* added at 1.5% to 2.5% to the feed of laying hens was as effective as synthetic pigments Lucantin<sup>®</sup>Red and Lucantin<sup>®</sup>Yellow in producing agreeable egg yolk colour.

Al-Harhi and El-Deek (2012) added brown marine algae *Sargassum dentifebium* at 3% and 6% to the diet of hens. The authors observed a significant increase in total carotene, lutein and zeaxanthin in eggs. Plasma and yolk cholesterol were significantly lower in treated groups.

*Chlorella*, a fresh water unicellular alga was cultivated heterotrophically and added at 1% and 2% to Hisex Brown laying hens. At higher dosing *Chlorella* doubled the concentration of total carotenoids, lutein, zeaxanthin, and  $\beta$ -carotene in egg yolk. The curves of total carotenoids reached the plateau during the fourth experimental week. The deposition of carotenoids significantly increased the colour of yolks using the Roche Yolk Colour Fan (Kotrbaček et al., 2013).

The red pepper is a rich source of carotenoids. Lokaewmanee et al. (2013) supplemented a basal diet of laying hens with red pepper at 0.5%. Compared with the control group, Roche yolk colour fan values were increased in all treated hens. Neither performance nor other egg quality parameters were influenced. The red pepper stimulated intestinal villi (villi height, villus area, cell area and cell mitosis in all intestinal segments).

### CONCLUDING REMARKS

Carotenoids are present in variable amounts in common ingredients of feed mixtures for poultry. Laying hens deposit dietary antioxidants preferentially in the egg and not in the body (Loetscher et al., 2014). Due to the high concentration of antioxidants in the yolk the oxidative stability of yolk lipids is relatively high. This may be further increased by supplementation of hen feeds with synthetic or natural carotenoids. The use of natural carotenoids is consistent with the preference of many consumers for natural products. The carry-over of carotenoids in the human food chain could also be important.

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