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EFFECT OF NATURAL AND SYNTHETIC CAROTENOIDS ON YOLK COLOUR AND OXIDATIVE STABILITY OF YOLK LIPIDS

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With the purpose of impact assessments of synthetic and natural carotenoids on the daily production of eggs and some indicators of their quality, two experiments were performed.

In order to evaluate some characteristics of yolk as colour and oxidative stability of lipids, the first experiment was conducted on two hundred and forty ISA Brown hens aged 25–39 weeks which were housed in cages (10 hens per 1 cage). Hens were fed by four different diets: control diet (the first group) and diets supplemented with synthetic carotenoids Carrophyl® Red and Carrophyll® Yellow at 20 and 15 mg/kg, respectively (the second group), with lutein powder extract at 250 mg/kg (the third group), and with 12.5 g/kg spray-dried Chlorella (the forth group). The control diet contained maize, wheat and soybean meal as the main ingredients. Eggs were collected daily. Yolk colour was determined using Yolk Colour Fan. Lipid peroxidation in yolks was determined in fresh eggs and eggs stored at 18° C for 4 weeks.

The second experiment was conducted with the purpose to compare effect of synthetic carotenoids, lutein and mustard (source of carotenoids) on daily output of eggs, yolk colour and contents of carotenoids in yolks. In the experiment, one hundred and sixty ISA Brown hens of age 20–34 weeks were housed in enriched cages. Control hens (the 1st group) were fed by a diet without carotenoids. Hens of the 2nd group were fed with a combination of Carophyll® Red and Carophyll® Yellow as it was described previously. Hens of the 3rd group were fed by a diet supplemented with lutein at 100 mg/kg. The 4th group received diet supplemented with 10 g/kg of meal from Brassica juncea (L.). The mustard meal contained lutein and zeaxanthin at 11.9 and 5.2 mg/kg, respectively. Sampling and yolk colour determination were the same as in the first experiment. The β-carotene contents of yolks were determined by HPLC.

Carotenoids had no effect on hen-day egg production. Both synthetic and natural carotenoids significantly increased the intensity of yolk colour. Carophylls, lutein and Chlorella significantly improved the oxidative stability of yolk lipids. It can be concluded that (i) lutein and Chlorella are alternatives to synthetic carotenoids, and (ii) the use of Chlorella is more advantageous from an economical point of view than that of lutein.

Key words: CAROTENOIDS, YOLK COLOUR, OXIDATIVE STABILITY, CHLORELLA

ВПЛИВ ПРИРОДНИХ І СИНТЕТИЧНИХ КАРОТИНОЇДІВ НА КОЛІР ЖОВТКА І ОКИСНЮВАЛЬНУ СТАБІЛЬНІСТЬ ЙОГО ЛІПІДІВ

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З метою оцінки впливу згодовування курам синтетичних і природних каротиноїдів на добову продукцію яєць та деякі характеристики їх якості було проведено два досліди.

Для оцінки таких характеристик жовтка, як колір і окиснювальна стійкість ліпідів, був проведений перший дослід на 240 курках кросу ISA BROWN віком 25–39 тижнів, які утримувалися у клітках (по 10 на кожну). Чотири групи курей годували згідно з чотирьома раціонами, а саме контрольним (I група) і дослідними раціонами з додаванням синтетичних каротиноїдів — червоного і жовтого Carrophyll® відповідно в кількості 20 і 15 мг/кг (II група), порошку лютеїнового екстракту в кількості 250 мг/кг (III група) і 12,5 г/кг висушеної розпиленням хлорели (IV група). Основними інгредієнтами раціону I (контрольної) групи були кукурудза, пшениця і соєва мука. Яйця відбирали щоденно. Колір жовтка *визначали за допомогою відповідного віяла для визначення інтенсивності забарвлення. Перекисне окиснення ліпідів у жовтку було визначено у свіжих яйцях і тих, що зберігалися при 18° С впродовж 4 тижнів.*

Другий дослід проводили з метою порівняння впливу синтетичних каротиноїдів, лютеїну і гірчиці (джерела каротиноїдів) на добову продукцію яєць, колір жовтка і вміст каротиноїдів. У досліді використовували сто шістдесят курок кросу ISA BROWN віком 20–34 тижні, що перебували у клітках відповідно до європейських стандартів. Кури І (контрольної) групи утримувалися на раціоні без каротиноїдів. Курей ІІ групи годували з додаванням комбінації синтетичних каротиноїдів — червоного і жовтого Carophyll®, як описано в першому досліді, ІІІ групи — лютеїну в кількості 100 мг/кг, IV групи — 10 г/кг гірчичної муки, яка містила лютеїн і зеаксантин відповідно у кількості 11,9 і 5,2 мг/кг. Відбір проб і визначення інтенсивності кольору жовтка проводили так само, як у першому досліді. Вміст β-каротину в жовтку визначали за допомогою високоефективної рідинної хроматографії.

Експериментальні результати показали, що каротиноїди не мали жодного впливу на добову продукцію яєць, тоді як синтетичні і природні їх представники вірогідно посилювали інтенсивність кольору жовтка яєць, а червоний і жовтий Carophyll® (синтетичні каротиноїди), лютеїн і хлорела вірогідно підвищували окиснювальну стабільність ліпідів жовтків яєць. Спираючись на дані досліджень, можна стверджувати, що лютеїн і хлорела — це альтернативні синтетичним каротиноїдам речовини, а більше переваг з економічної точки зору має використання хлорели (Chlorella), ніж лютеїну.

Ключові слова: КАРОТИНОЇДИ, КОЛІР ЖОВТКА, ОКИСНЮВАЛЬНА СТАБІЛЬНІСТЬ, CHLORELLA

ВЛИЯНИЕ ПРИРОДНЫХ И СИНТЕТИЧЕСКИХ КАРОТИНОИДОВ НА ЦВЕТ ЖЕЛТКА И ОКИСЛИТЕЛЬНУЮ СТАБИЛЬНОСТЬ ЕГО ЛИПИДОВ

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С целью оценки влияния скармливания курам синтетических и природных каротиноидов на суточную продукцию яиц и некоторые характеристики их качества были проведены два эксперимента.

Для оценки таких характеристик желтка, как цвет и окислительная устойчивость липидов, был проведен первый эксперимент на 240 курах кросса ISA BROWN в возрасте 25–39 недель, которые содержались у клетках (по 10 на кардую). Кур кормили согласно четырех рационов: контрольного (I группа) и экспериментальных рационов с добавками синтетических каротиноидов — красного и желтого Carrophyll® соответственно в количестве 20 и 15 мг/кг (II группа), порошка лютеинового экстракта в количестве 250 мг/кг (III группа) и 12,5 г/кг высушенной распылением хлореллы (IV группа). Рацион I (контрольной) группы содержал кукурузу, пшеницу и соевую муку в качестве основных ингредиентов. Яйца отбирали ежедневно. Цвет желтка определяли с помощью соответствующего веера для определения интенсивности цвета. Перекисное окисление липидов в желтке было определено в свежих яйцах и тех, которые хранились при 18° С в течение 4 недель.

Второй эксперимент проводили с целью сравнения влияния синтетических каротиноидов, лютеина и горчицы (источника каротиноидов) на суточную продукцию яиц, цвет желтка и содержание каротиноидов. В опыте использовали 160 кур кросса ISA BROWN в возрасте 20–34 недели, находящихся в клетках соответственно европейским стандартам. Куры I (контрольной) группы содержались на рационе без каротиноидов. Кур II группы кормили с добавлением комбинации синтетических каротиноидов — красного и желтого Carophyll®, как описано в первом опыте, III группы — лютеина в количестве 100 мг/кг, IV группы — 10 г/кг горчичной муки, которая содержала лютеин и зеаксантин соответственно в количестве 11,9 и 5,2 мг/кг. Отбор проб и определение интенсивности цвета желтка проводили так же, как в первом опыте. Содержание β-каротина в желтке определяли при помощи высокоэффективной жидкостной хроматографии.

Экспериментальные результаты показали, что каротиноиды не имели никакого влияния на суточную продукцию яиц и в то же время синтетические и природные их представители достоверно усиливали интенсивность цвета желтка яиц, а красный и желтый Carophyll® (синтетические каротиноиды), лютеин и хлорелла (Chlorella) достоверно повышали окислительную стабильность желтков *яиц. Данные исследований свидетельствуют, что лютеин и хлорелла — это альтернативные вещества синтетическим каротиноидам, а с экономической точки зрения использование хлореллы (Chlorella) имеет больше преимуществ, чем использование лютеина.*

Ключевые слова: КАРОТИНОИДЫ, ЦВЕТ ЖЕЛТКА, ОКИСЛИТЕЛЬНАЯ СТАБИЛЬНОСТЬ, CHLORELLA

Carotenoids are yellow, orange, and red pigments synthesized by plants and microorganisms. In the poultry industry, carotenoids are routinely added to diets of laying hens to obtain optimum pigmentation of the egg yolk and increase oxidative stability of yolk lipids. Carotenoids may serve as a precursor of vitamin A and also have immunomodulatory functions [1]. To overcome the shortage of natural carotenoids, synthetic carotenoids were prepared. Canthaxanthin is the preferred synthetic red xanthophyll in poultry farming available as Carophyll® Red (DSM Nutritional Products, Switzerland) or Lucantin® Red (BASF, Germany). The preferred yellow xanthophyll is β-apo-8´-carotenoic acid ethyl ester available as Carophyll® Yellow and Lucantin® Yellow. Carotens and β-cryptoxanthin are provitamins A which can be converted to retinol in the body. Also lycopene, a bright red carotenoid, found in tomatoes was tested in several studies. Ševčíková et al. [2] investigated the effect of lycopene supplementation on lipid profile and meat quality of broiler chickens, Englmaierová et al. [3] reported the effect of lycopene on quality and oxidative stability of chicken meat, and Xue et al. [4] reported the effect of a lycopene-containing tomato by-product on the yolk colour and lycopene content of eggs. Lutein is an orange xanthophyll occurring in plants usually with carotens. Jang et al. [5] showed that both commercial lutein and lutein-containing extract of spinach significantly increased the egg yolk lutein content and yolk colour.

This paper summarizes the results of two experiments aimed at improvement of oxidative stability and colouring of yolks using natural and synthetic carotenoids.

Materials and methods

In the first experiment, lutein, dried *Chlorella* and synthetic carotenoids were compared. Two hundred and forty ISA Brown hens aged 25–39 weeks were housed in enriched cages, 10 hens per cage. There were four dietary treatments. The control diet (the first group) contained maize, wheat and soybean meal as the main ingredients. The hens of the second group obtain a combination of Carophyll® Red and Carophyll® Yellow at 20 and 15 mg/kg, respectively. The hens of the third group received diet supplemented with Lutein powder extract (Alchimica, Prague, Czech Republic) at 250 mg/kg. Diet of hens of the fourth group was supplemented with 12.5 g/kg spray-dried *Chlorella*, cultivated autotrophically in the Institute of Microbiology (Třeboň, Czech Republic).

Eggs were collected daily. Yolk colour was determined by using Yolk Colour Fan (DSM Nutritional Products). Lipid peroxidation in yolks was determined in fresh eggs and eggs stored at 18°C for 4 weeks. The thiobarbituric acid method by Piette and Raymond [6] was used. Thiobarbituric acid-reactive substances (TBARS) were expressed as mg of malondialdehyde per kg. The data were analyzed by one-way ANOVA (SAS, version 9.2).

In the second experiment, synthetic carotenoids, lutein and mustard were compared. Mustard seed is a potential source of carotenoids. One hundred and sixty ISA Brown hens, 20–34 weeks of age, were housed in enriched cages, conforming to the EU Directive 1999/74/EC. The hens were assigned to one of 4 dietary treatments. Hens of the 1st group (control) were fed br a diet without carotenoids. Hens of the 2nd group were fed a combination of Carophyll® Red and Carophyll® Yellow as described previously, hens of the 3rd group were fed by diet supplemented with lutein at 100 mg/kg. The last experimental (the 4th) group received diet supplemented with 10 g/kg of meal from *Brassica juncea* (L.). The mustard meal was obtained from Oseva Pro, Ltd. (Opava, Czech Republic). The mustard meal contained lutein and zeaxanthin at 11.9 and 5.2 mg/kg, respectively. For sampling and yolk colour determination see the first experiment. The β-carotene contents of yolks were determined in accordance with the standard EN 12823-2 [7]. The HPLC instrument (VP series; Shimadzu, Kyoto, Japan) equipped with a diode-array detector was used. The data were analyzed statistically using one-way ANOVA.

Results and discussion

Results of the first experiment are presented in *Table 1* and *Fig. 1*. Neither synthetic nor natural carotenoids significantly influenced hen-day egg production. Carophylls, lutein and *Chlorella* significantly increased the yolk colour. The strongest effect was that of lutein. Carophylls were better colouring agents than dried *Chlorella*. All supplements significantly increased the oxidative stability of yolk lipids, expressed as malondialdehyde level.

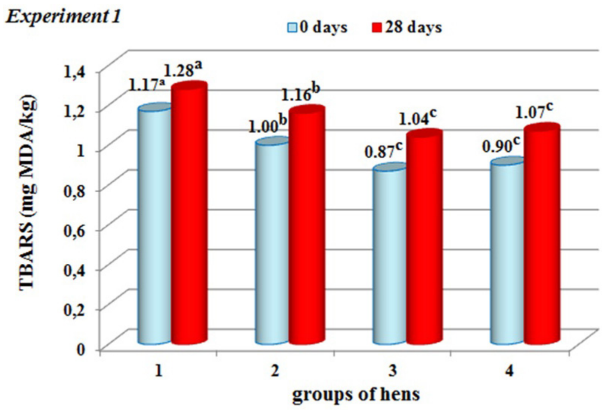

Fig. 1. Oxidative stability of fresh eggs and eggs stored for 28 days, results of the 1st experiment ($^{a\text{-}c}P$<0.05; MDA — malondialdehyde)

Table 1

Daily output of eggs, yolk colour (results of the 1st experiment)

Indicators	Groups of hens			
	1	2	3	4
Hen-day egg production (%)	92.8	93.4	93.9	93.8
Yolk colour (DSM Fan)	6.4^{d}	10.7^{b}	13.1^{a}	8.9^{c}

Note: $^{a\text{-}d}$ P<0.05

Results of the second experiments are shown in *Table 2* and *Fig. 2*. The source of carotenoides, either synthetic or natural, did not influence the hen-day egg production. Colour of yolks in hens fed the mustard meal was comparable to that of yolks in hens fed lutein at 100 mg/kg and significantly higher than in control hens. Feed supplementation with Carophylls and lutein significantly increased concentrations of β-carotene, lutein and zeaxanthin in egg yolks. The contents of carotenoids in yolks of hens fed the mustard meal were not significantly different from the control.

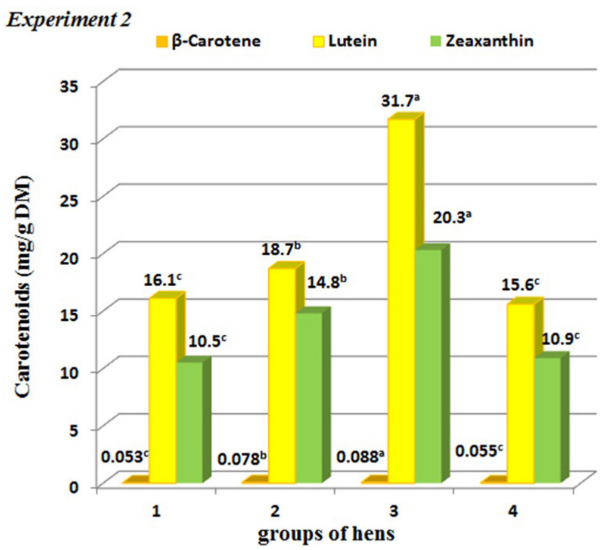

Fig. 2. Contents of carotenoids in yolks, results of the 2nd experiment ($^{a\text{-}c}$ P<0.05; DM — dry matter)

Table 2

Daily output of eggs and yolk colour (results of the 2nd experiment)

Indicators	Groups of hens			
	1	2	3	4
Hen-day egg production (%)	89.0	90.0	85.5	91.8
Yolk colour (DSM Fan)	7.7^{c}	11.8^{a}	8.4^{b}	8.3^{b}

Note: $^{a\text{-}c}$ P<0.05

All carotenoids are efficient antioxidants that alleviate the oxidative stress. Eggs are excellent vehicles for the carry-over of carotenoids in the human food chain [8]. Lutein and its isomer zeaxanthin accumulate in the macular region of the retina and protected eyes against the development of cataract and macular degeneration [9]. Lutein-enriched eggs have greater lutein bioavailability for humans than commercial supplements [10].

The egg yolk colour is one of the main characteristics of egg quality. To satisfy consumer's demand, synthetic carotenoids are used as colouring agents. These carbonyl derivatives include ethyl ester of β-apo-8′-carotenoic acid, canthaxanthin and astaxanthin. However, some harmful side effects of the canthaxanthin application have been described in the literature [11, 12], thus the maximum amount of canthaxanthin should be 8 mg/kg feed. Lutein and spray-dried algae *Chlorella* thus represent a suitable alternative to synthetic carotenoids. From an economical point of view, the use of *Chlorella* is more advantageous than that of lutein, which is rather expensive. Our results are consistent with those of Kotrbáček et al. [13] who supplemented a diet of laying hens with 1 % or 2 % of dry disintegrated *Chlorella*. Egg yolk deposition of total carotenoids was significantly ($P<0.01$) increased by 46 % and 119 %, also the yolk colour was increased. Another natural source of carotenoids, usable in the nutrition of layers, is the extract of flowers of marigold (*Tagetes erecta*), available commercially as Avizant® Yellow 20 HS (Lohmann Animal Health, Cuxhaven, Germany).

Conclusions

Since carotenoids are precursors of vitamin A and also have immunomodulatory functions that's why they are routinely added to diets of laying hens to obtain optimum pigmentation of the egg yolk and increase oxidative stability of yolk lipids in the poultry industry. It's known that synthetic carotenoids were prepared to overcome the shortage of natural carotenoids; therefore, there are important investigations of synthetic and natural carotenoids on health of hens and eggs quality. From our data, it could be concluded that lutein and *Chlorella* are alternatives to synthetic carotenoids, and the use of *Chlorella* is more advantageous from an economical point of view than that of lutein.

Perspectives of future research. Future research will be focused at studying the impact of synthetic and natural carotenoids on the organism of hens, production of eggs and their characteristics for implementation in production. The extract of flowers of marigold (another natural source of carotenoids) would be next step of comparative study of effect of natural carotenoids on organism of hens. There is also a perspective of important investigation of influence of supplementation of the natural carotenoids on lipid profile and chicken meat quality.

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