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Utility of nettle (*Urtica dioica*) in layer diets as a natural yellow colorant for egg yolk

Y. Loetscher, M. Kreuzer*, R.E. Messikommer

ETH Zurich, Institute of Agricultural Sciences, Universitaetstrasse 2, 8092 Zurich, Switzerland

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ABSTRACT

Yolk color is an important quality trait of eggs. Natural pigment sources are preferred by consumers. Synthetic pigments are banned in some production systems. As nettle (*Urtica dioica*) addition was found to substantially increase broiler skin yellowness, it was hypothesized to be a potent natural yolk coloring feed component. Therefore, the pigmentation by nettle and possible side-effects on performance, egg quality and antioxidant properties were tested in a 4-week experiment with 40 individually caged H&N Nick Brown layers (70 weeks of age). A basal feed mixture low in pigments and tocopherol was designed. Two weeks prior to the experiment, all animals received this basal mixture plus synthetic pigments (25 mg/kg Carophyll® Yellow, 15 mg/kg Carophyll® Red). Thereafter, eight animals each received diets either with 0, 6.25, 12.5 or 25 g nettle per kg put into the basal mixture or a control diet containing synthetic pigments like the pre-experimental diet and extra 40 mg/kg of α-tocopherylacetate. In detail, nettle was supplied by two independent batches to four animals per dosage each. Performance was assessed during 4 weeks and feed and egg samples were collected in the last week of the experiment and analyzed for various variables with a focus on color and antioxidant traits. By applying different statistical models, a comparison with the control animals, batch differences and nettle dosage effects were evaluated. Yolk yellowness (b^*) increased with nettle addition depending on dosage and batch, yet was equally effective as synthetic pigmentation (29.4) in all investigated cases (avg. 30.3). The score according to the DSM-Yolk Color Fan increased from 1.7 in the non-supplemented group to 4.2 (6.25 g nettle A/kg) and up to 6.5 (25 g nettle B/kg). This increase depended on dose and batch. Due to the lack of red pigments in nettle, scores were still lower than with the control treatment (9.5). The development of thiobarbituric acid reactive substances, monitored over 12 weeks in lyophilized yolk powder, was not affected by batch or nettle concentration. However, yolk from nettle fed hens, especially from one batch, was richer in tocopherols with increasing dosage though being far from that found in the tocopherol supplemented hens. There was no substantial influence of nettle supplement or batch on laying performance and general egg quality. Nettle supplementation of layer diets is therefore considered as an effective means to naturally achieve the desired yolk yellowness, and this without risking unfavorable side-effects.

Abbreviations: ADFI, average daily feed intake; ADFom, acid detergent fiber exclusive of residual ash; AIA, acid insoluble ash; aNDFom, neutral detergent fiber assayed with a heat stable amylase and exclusive of residual ash; ME, metabolizable energy; RAF, Rancimat antioxidant factor; RSA, radical scavenging activity; TBARS, thiobarbituric acid reactive substances; YCF, DSM-Yolk Color Fan.

* Corresponding author. Tel.: +41 44 632 59 72; fax: +41 44 632 11 28.
E-mail address: michael.kreuzer@inw.agrl.ethz.ch (M. Kreuzer).

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1. Introduction

Color is an important quality trait of foods, as it has an impact on the consumers' perception of quality and intensity of aroma and flavor (Christensen, 1983) and their purchase decision. Most consumers associate yolk color to age and health status of the animal and to the quality of eggs and egg products, but objectively this is not the case. Therefore, producers aim at rather intense and uniform pigmentation by appropriate feeding measures.

A subclass of the carotenoids, the xanthophylls, is responsible for egg yolk pigmentation (Hencken, 1992), and the desired color is achieved by a combination of yellow and red xanthophylls. Many carotenoids are supposed to be antioxidants. It has even been proposed that their incorporation to the egg yolk by the hen happens in favor of the developing chicken (Surai, 2002). In combination with α-tocopherylacetate, a synergistic increase in antioxidant activity has been demonstrated (Leibovitz et al., 1990; Palozza and Krinsky, 1992). Since animals are not able to synthesize carotenoids de novo, deposition in tissue and eggs depends on supplementation since the amount of yellow pigments present in an unsupplemented diet is too low. However, the use of synthetic additives is not allowed in certain forms of production, especially organic farming (Codex Alimentarius, 2013), and discouraged in others. Additionally, consumers' preferences generally tend toward natural rather than synthetic feed additives.

The most common natural sources of yellow and red xanthophylls used for yolk coloring originate from marigold (*Tagetes erecta*) petals or extracts thereof (Lokaewmanee et al., 2011) and from red pepper (*Capsicum* ssp.) (Santos-Bocanegra et al., 2004), respectively. Maize and alfalfa meal also contain xanthophylls; this, however, at much lower and more inconsistent levels (Nys, 2000). Alternatively, nettle (*Urtica dioica*) may also have particular egg yolk coloring properties because its supplementation resulted in a significantly increased yellowness of broiler skins in a previous experiment (Loetscher et al., 2013). Nettle is indeed rich in yellow coloring xanthophylls like lutein and lutein isomers (Guil-Guerrero et al., 2003). Because marigold is native to the tropics, nettle would be an interesting alternative as natural colorant, which can be locally grown in temperate climates. At current prices, this would be economically feasible, but only in case nettle turns out to be as efficient as a colorant as expected. The yolk coloring efficiency of nettle still needs to be demonstrated, because bioavailability and, with that, transfer rate of pigments to the yolk differs greatly between sources and types of xanthophylls (Karunajeewa et al., 1984). In addition, despite the prevalence of xanthophylls, there was even a slightly higher susceptibility to oxidation of the meat when nettle was provided at 25 g/kg feed to broilers (Loetscher et al., 2013). Bearing in mind that an excessive amount of antioxidants may turn pro-oxidant (Ristow and Schmeisser, 2011), it is possible that lower nettle dosages still could act as an antioxidant.

In the present study, the following hypotheses were tested: (i) egg yolk yellowness can be increased with nettle supplementation of the layer's diet to an extent which is similar to that of feed supplemented with common dosages of synthetic pigments; (ii) the dose–response of increasing nettle levels is linear; (iii) the between-batch differences are small enough to allow a generalization of the effects. Possible side-effects of nettle on performance, general egg quality and antioxidant activity exhibited directly in the yolk were also followed in detail.

2. Materials and methods

In the experiment, 40 H&N Brown Nick (H&N International GmbH, Cuxhaven, Germany) laying hens which were 70 weeks old were kept in individual cages with a size of 80 cm × 80 cm × 80 cm. All cages were equipped with nest, perch and sand bath. Water and feed (mealy form) was provided at *ad libitum* access. Air temperature was maintained at 20 °C. The animals were provided with artificial light during 14 h/day. The animal experiment was approved by the cantonal authority (Kantonales Veterinäramt, Zurich, Switzerland).

2.1. Experimental design and diets

A basal feed mixture with low pigment and antioxidant levels was designed, and no pigments or tocopherols were added. Its ingredient composition was (g/kg): wheat, 340; soybean meal, 165; rice, broken, 139; sorghum (milocorn), 120; limestone grit, 49; calcium carbonate, 40; soybean oil, 37; potato protein, 30; milling by-products, 22; oat husks, 20; celite (no. 545, acid-washed diatomaceous earth, Schneider Dämmtechnik, Winterthur, Switzerland), 15.5; di-calcium phosphate, 11; mineral-vitamin premix, 5; sodium bicarbonate, 3; DL-methionine, 2; sodium chloride, 1.5. This diet was calculated to contain per kg as fed: 11.6 MJ metabolizable energy (ME), 168 g crude protein, 8.30 g lysine, 7.74 g methionine & cysteine, and 38.7 g calcium. The mineral-vitamin premix provided per kg of feed: Ca, 1.25 g; Mn, 80 mg; Zn, 60 mg; Mg, 50 mg; Fe, 50 mg; Cu, 6 mg; I, 310 μg; Se, 200 μg; retinol, 12,500 IU; cholecalciferol, 3000 IU; choline, 100 mg; betaine, 99.9 mg; niacin, 40.5 mg; pantothenic acid, 15 mg; riboflavin, 5 mg; pyridoxine, 3.95 mg; menadione, 2.6 mg; thiamine, 2 mg; folic acid, 1.49 mg; biotin, 200 μg; cyanocobalamin, 20 μg. This basal mixture was supplemented with nettle from two different batches (A and B) at dosages of 0, 6.25, 12.5, or 25 g/kg each. The highest dosage was equivalent to the level tested before in broilers (Loetscher et al., 2013). Additionally, a control group received the basal feed mixture supplemented with 40 mg/kg α-tocopherylacetate (Rovimix E 50, DSM, Basle, Switzerland) and 25 mg/kg Carophyll® Yellow and 15 mg/kg Carophyll® Red (DSM, Basle, Switzerland), the latter aiming to result in a Yolk Color Fan (YCF) score of 10 (control diet).

Eight hens each were allocated in a balanced manner considering laying performance to the five main treatments, *i.e.* the four levels of nettle and the control. With nettle, four hens each received diets containing batch A and the other four batch