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Effects of flavonoids dietary supplementation on egg yolk antioxidant capacity and cholesterol level

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Abstract

An experiment was conducted to examine the effects of supplementing laying hen feed with different levels of hesperidin or naringin, bioflavonoids that are abundant and inexpensive by-products of citrus cultivation, on the yolk antioxidant capacity and cholesterol level. Seventy-two laying hens, approximately twelve months old, were assigned into 6 experimental groups of twelve hens each. One of the groups served as control (C) and was given a commercial basal diet, without bioflavonoid supplementation, whereas the other five groups were given the same diet further supplemented with hesperidin at low (750mg/kg of feed) (H1) or high (1500mg/kg) (H2) concentration or naringin at low (750mg/kg) (N1) or high (1500mg/kg) (N2) concentration or α -tocopheryl acetate (200mg/kg) (E). Measurements of yolk antioxidant capacity were performed on 8 eggs from each dietary group, at 0, 4, 7, 28 and 63 days after the beginning of the experiment. Yolk cholesterol level was determined on the final day (63th) of the experimental period. Oxidative stability of egg yolk, expressed as ng MDA/g yolk, was significantly improved in the hesperidin and naringin groups even from the first four days of the supplementation period ($P < 0.001$). However, no flavonoids effect on yolk cholesterol level (mg/g) was observed. Antioxidant properties of flavonoids seem to be a promising natural agent for improving the health status and the shelf life of laying hens' egg.

Keywords: bioflavonoids; yolk; antioxidant capacity; cholesterol level

Introduction

The increased awareness of consumers towards a diet rich in natural, safe and health-promoting ingredients has led to the search of alternative sources which may be used in the food and feed industry because of their valuable nutritional properties (Wenk, 2003). In poultry, interest have been triggered in improving the quality and the antioxidant properties of eggs with the intention to produce a high-quality functional food. The use of natural antioxidants appears as a great alternative, since it can prolong the shelf life and increase the acceptability of eggs and their economic value in the marketplace (Botsoglou *et al.*, 2005).

Eggs have a high nutritional value and contain a variety of necessary components for the maintenance and the normal function of the human organism. Feeding strategies are usually used to increase the n-3 fatty acid content of eggs by enriching poultry diets with polyunsaturated



fatty acids, constituents that increase the degree of unsaturation and the susceptibility of eggs to the oxidative deterioration. Lipid oxidation by free radicals is one of the primary mechanisms of quality deterioration in eggs. It is initiated in the highly-unsaturated fatty acid fraction of membrane phospholipids, leading to the production of hydroperoxides, which are susceptible to further oxidation or decomposition to secondary reaction products such as short-chain aldehydes, ketones and other oxygenated compounds that may adversely affect lipids, pigments, proteins, carbohydrates, vitamins and the overall quality by causing loss of flavour, colour and nutritive value and limiting shelf-life (Cherian et al., 1996).

Dried citrus pulp is an abundant and inexpensive by-product of citrus cultivation and it is produced after extraction of the juice from citrus fruits and drying of the residues. The final product is a mixture of peel, inside portions and culled fruits of the citrus family, rich in energy, fiber and calcium. Fibers from citrus fruits have an additional advantage over dietary fibers from other sources due to the presence of associated bioactive compounds (i.e. bioflavonoids) (Gorinstein et al., 2001). Hesperidin and naringin belong to flavanones, a sub-group of the flavonoids, and are naturally occurring polyphenolic compounds widely distributed in the plant kingdom as secondary metabolites. They usually contain one or more aromatic hydroxyl groups, which actively scavenge free radicals and are responsible for the respective antioxidant properties (Pietta, 2000).

The combination of increased disposal costs in many parts of the world with the antioxidant properties of citrus by-products have increased interest in their utilization as alternative feeds in animal production. The objective of the present study was therefore the evaluation of the effects of different levels of hesperidin or naringin dietary supplementation on egg yolk antioxidant capacity and cholesterol level.

Materials and Methods

Seventy-two brown (Brown-Classic) Lohmann individually-caged laying hens (12 months old) were randomly assigned into 6 equal treatment groups (12 hens each). One of the groups served as a control (C) and was given a commercial basal diet, whereas the other five groups were given the same diet further supplemented with hesperidin (Sigma-Aldrich, Co., USA) at 0.75 g/kg of feed (H1), or 1.5 g/kg (H2), or naringin (Alfa Aesar GmbH & Co KG, Germany) at 0.75 g/kg (N1), or 1.5 g/kg (N2) or α -tocopheryl acetate (DSM Nutritional Products, Greece) at 0.2mg/kg (E) for 63 days. Water was provided *ad libitum* throughout the experimental period and the light regimen was 16 h of continuous light per day. Each hen consumed approximately 110 g feed/day, respectively. The diet consisted of a commercial concentrate mixture containing maize (54 %), wheat (5 %), soybean meal (24.6 %), wheat bran (4.6 %), soybean oil (1.2 %), limestone (8.9 %), monocalcium phosphate (0.9 %), sodium chloride (0.24 %), sodium bicarbonate (0.27 %), methionine (0.1 %), choline (0.06 %) and a vitamins & minerals premix (0.1 %). Metabolizable energy and crude protein were 11.48 MJ and 175 g per kg feed, respectively.



Yolk oxidative stability was assessed by using the malondialdehyde (MDA) assay on 8 eggs collected from each dietary group at 0, 4, 7, 28 and 63 days after the beginning of bioflavonoids dietary supplementation. Measurements of yolk cholesterol were performed on 8 eggs collected from each dietary group, on day 63 of the experiment. The methods used in the present experiment were in accordance with the national legislation and the guidelines of the Research Ethics Committee of the Department of Animal Science and Aquaculture of the Agricultural University of Athens.

Yolk cholesterol was determined following the method described by Pasin et al. (1998). In detail, 3 g of egg yolk was diluted with 27 ml NaCl (20g/kg), stirred for 2 hours using a magnetic stirrer, and 1 ml of the above solution was further diluted with 9 ml NaCl (20 g/kg). Cholesterol was measured photometrically at 540 nm in a spectrophotometer (Hitachi U3010 Spectrophotometer) by using a commercial cholesterol reagent kit (Biosis commercial kits; Athens, Greece).

Lipid oxidation was assessed on the basis of the malondialdehyde (MDA) formed during storage, a secondary lipid oxidation product formed by hydrolysis of lipid hydroperoxides. In the present study, yolk MDA concentration was determined by using a selective third-order derivative spectrophotometric method (Botsoglou *et al.*, 1994). Derivative versus conventional spectrophotometry was adopted because it offers improved sensitivity, specificity and reliability of the measurements, since it eliminates potential interferences from other reactive compounds.

Data were subjected to analysis of variance with nutritional treatment as fixed effect using a general linear model (GLM) of SAS software (SAS Institute, 2005). Mean differences were tested at 0.05 significance level and results are presented as means \pm standard error.

Results and Discussion

Results presented in Table 1 showed that bioflavonoids dietary supplementation for 63 days did not influence yolk cholesterol values ($P > 0.05$). According to previous implemented studies, yolk cholesterol content decreases after hesperetin or naringenin supplementation in laying hens, as a result of the inhibition of the key enzymes in the cholesterol synthesis, HMG-CoA reductase (Lien *et al.*, 2008; Ting *et al.*, 2011). On the other hand, no effect on yolk cholesterol level was observed after the incorporation of hesperidin into the hen diet for 28 days (Goliomytis *et al.*, 2014). Discrepancies among previously implemented studies may be attributed to the different mode of action of the various substances (i.e. hesperetin or hesperidin) and their dosages.

Table 1. Effect of 63-d dietary hesperidin or naringin or α -tocopheryl acetate supplementation on egg yolk cholesterol level (mg/g) (means \pm se)

	Experimental group					Standard error	P value	
	C	H1	H2	N1	N2			E
Yolk cholesterol (mg/g)	5.83	5.86	5.85	5.82	5.76	5.75	0.20	0.998

C, 0 g/kg feed; H1, 0.75 g hesperidin per kg feed; H2, 1.5 hesperidin per kg feed; 0.75 g naringin per kg feed; H2, 1.5 naringin per kg feed; E, 0.2 g α -tocopheryl acetate per kg feed.



As it is presented in Table 2, dietary flavonoids supplementation improved yolk oxidative stability even from the fourth day of the experiment, even at the low concentration levels (0.75g/kg) ($P < 0.001$). Moreover, oxidation values of the bioflavonoids supplemented groups were similar to that of the α -tocopheryl acetate supplemented group. In a recent implemented study by Goliomytis *et al.* (2014), inclusion of hesperidin in laying hens' diets for 1 week, even at 1 g/kg, have significantly reduced yolk oxidation values. Moreover, Lien *et al.* (2008) and Ting *et al.* (2011), who investigated the antioxidant activity on hens' blood samples, found that serum superoxide dismutase (SOD) level was relatively high after hesperetin and naringenin supplementation (0.5-4 g/kg) resulting in a reduced superoxide anion level. The above results indicated that hesperetin and naringenin can possibly terminate chain radical reaction by donating hydrogen atoms to the free radicals; an action comparable to vitamin E (Van Acker *et al.*, 2000).

Table 2. Effect of dietary hesperidin or naringin or α -tocopheryl acetate supplementation on egg yolk oxidative stability (ng MDA/g yolk) in laying hens by day (means \pm se) (higher levels of MDA indicate higher rates of lipid oxidation)

Effect	Duration of supplementation (days)				
	0	4	7	28	63
C	3.20	3.14 ^a	3.11 ^a	2.69 ^a	2.81 ^a
H1	2.74	2.35 ^b	2.34 ^b	1.88 ^b	1.89 ^b
H2	2.93	2.29 ^b	1.93 ^b	1.83 ^b	1.81 ^b
N1	2.84	2.07 ^b	1.89 ^b	1.86 ^b	1.82 ^b
N2	2.74	2.19 ^b	2.05 ^b	1.85 ^b	1.75 ^b
E	2.94	2.34 ^b	2.00 ^b	1.74 ^b	1.70 ^b
Standard error	0.20	0.17	0.15	0.13	0.15
P value	0.456	0.001	<0.001	<0.001	<0.001

C, 0 g/kg feed; H1, 0.75 g hesperidin per kg feed; H2, 1.5 hesperidin per kg feed; 0.75 g naringin per kg feed; H2, 1.5 naringin per kg feed; E, 0.2 g α -tocopheryl acetate per kg feed. ^{a,b} Means within columns sharing no common superscript significantly differ ($P < 0.05$)

Results of the present study revealed that when hesperidin or naringin are incorporated in hens' diet, lipid oxidation values are decreased and as a result egg quality is improved and shelf-life is increased. However, further experimentation is warranted to elucidate its exact action in hens' metabolism.

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