



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

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## Introduction (1)

- 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 (PUFAs) → increase in the degree of unsaturation and the susceptibility of eggs to the oxidative deterioration

## Introduction (2)

- Lipid oxidation is initiated in the highly-unsaturated fatty acid fraction of membrane phospholipids
- Formation of hydroperoxides → susceptible to further oxidation or decomposition to secondary reaction products → short-chain aldehydes, ketones and other oxygenated compounds → adversely affect lipids, pigments, proteins, carbohydrates, vitamins and the overall quality by **causing loss of flavour, colour and nutritive value and limiting shelf-life**

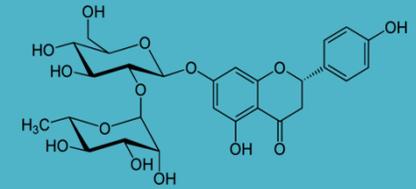
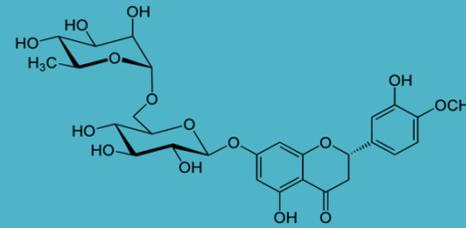
## Introduction (3)

- Natural antioxidants:
  - ✓ are regarded as compounds capable of delaying, retarding or preventing oxidation processes by scavenging initial radicals, decomposing primary products of oxidation to non-radical species and breaking chains to prevent continued hydrogen abstraction from substrates
  - ✓ preserve the integrity of cell membranes by preventing the oxidation of membrane phospholipids
  - ✓ appear as a great alternative, since their use can also prolong the shelf life and increase the acceptability of eggs and their economic value in the marketplace

# Citrus Pulp and Bioflavonoids

- Dried citrus pulp is an abundant and inexpensive by-product of citrus cultivation → 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 → presence of associated bioactive compounds (i.e. bioflavonoids)
- Flavonoids, such as hesperidin and naringin, are naturally occurring polyphenolic compounds widely distributed in the plant kingdom as secondary metabolite
- Contain one or more aromatic hydroxyl groups, which actively scavenge free radicals and are responsible for the respective antioxidant properties

# Hesperidin and Naringin



- Flavanone glycosides found abundantly in citrus fruits
- Hesperidin and naringin decrease cholesterol level and blood pressure, have anti-inflammatory effects and exhibit pronounced anticancer activity against some selected human carcinoma cell lines
- Hesperidin and naringin are deglycosylated by intestinal microflora in the colon to produce the active aglycones hesperetin and naringenin that are then absorbed in the gut and subsequently glucuronidated and circulate in plasma

## Objective of the study

- Increased disposal costs in many parts of the world & the antioxidant properties of citrus by-products → 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

## Material and Methods (1)

- 72 brown (Brown-Classic) Lohmann individually-caged laying hens (12 months old) were randomly assigned into 6 equal treatment groups for 63 days:



- ✓ control (C) was given a commercial basal diet,
- ✓ supplemented with hesperidin at 0.75 g/kg (H1) or 1.5 g/kg (H2),
- ✓ supplemented with naringin at 0.75 g/kg (N1) or 1.5 g/kg (N2),
- ✓ supplemented with  $\alpha$ -tocopheryl acetate at 0.2 g/kg (E)

## Material and Methods (2)

- 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 and 15 & 30 days after storage at room temperature and 60 & 120 days after refrigerated storage at 4°C
- MDA is a secondary lipid oxidation product formed by hydrolysis of lipid hydroperoxides and was determined by using a selective third-order derivative spectrophotometric method (Botsoglou et al., 1994)
- Derivative spectrophotometry was adopted because it offers improved sensitivity, specificity and reliability of the measurements, since it eliminates potential interferences from other reactive compounds

## Material and Methods (3)

- Measurement of yolk cholesterol was performed on 8 eggs collected from each dietary group on day 62 of the experiment, following a method described by Pasin et al. (1998)
- 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
- Adjustment of P-values was based on Bonferroni correction

# Results (1)

*Effect of dietary hesperidin or naringin or  $\alpha$ -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)*

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

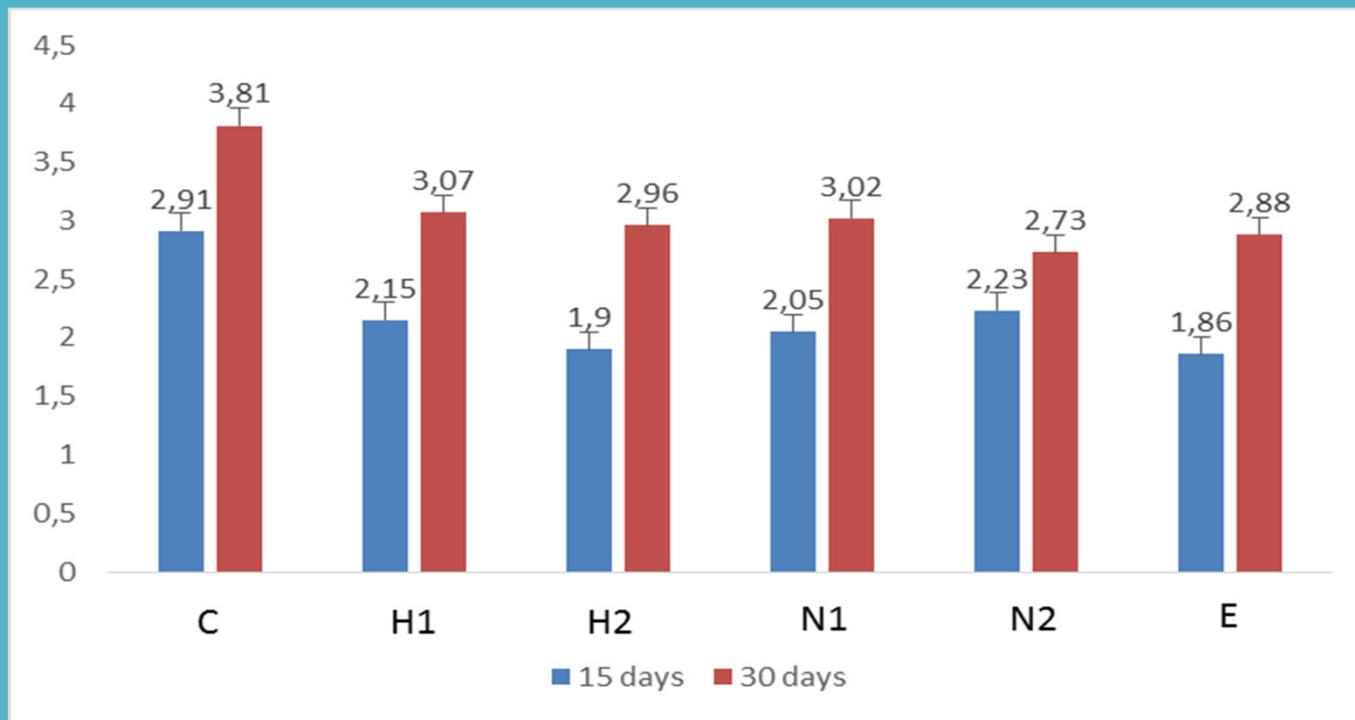
C, 0 g/kg feed; H1, 0.75 g hesperidin per kg feed; H2, 1.5 hesperidin per kg feed; N1, 0.75 g naringin per kg feed; N2, 1.5 naringin per kg feed; E, 0.2 g  $\alpha$ -tocopheryl acetate per kg feed.

<sup>a,b</sup> Means within columns sharing no common superscript significantly differ (P<0.001)

<sup>A,B,C</sup> Means within rows sharing no common superscript significantly differ (P<0.01)

## Results (2)

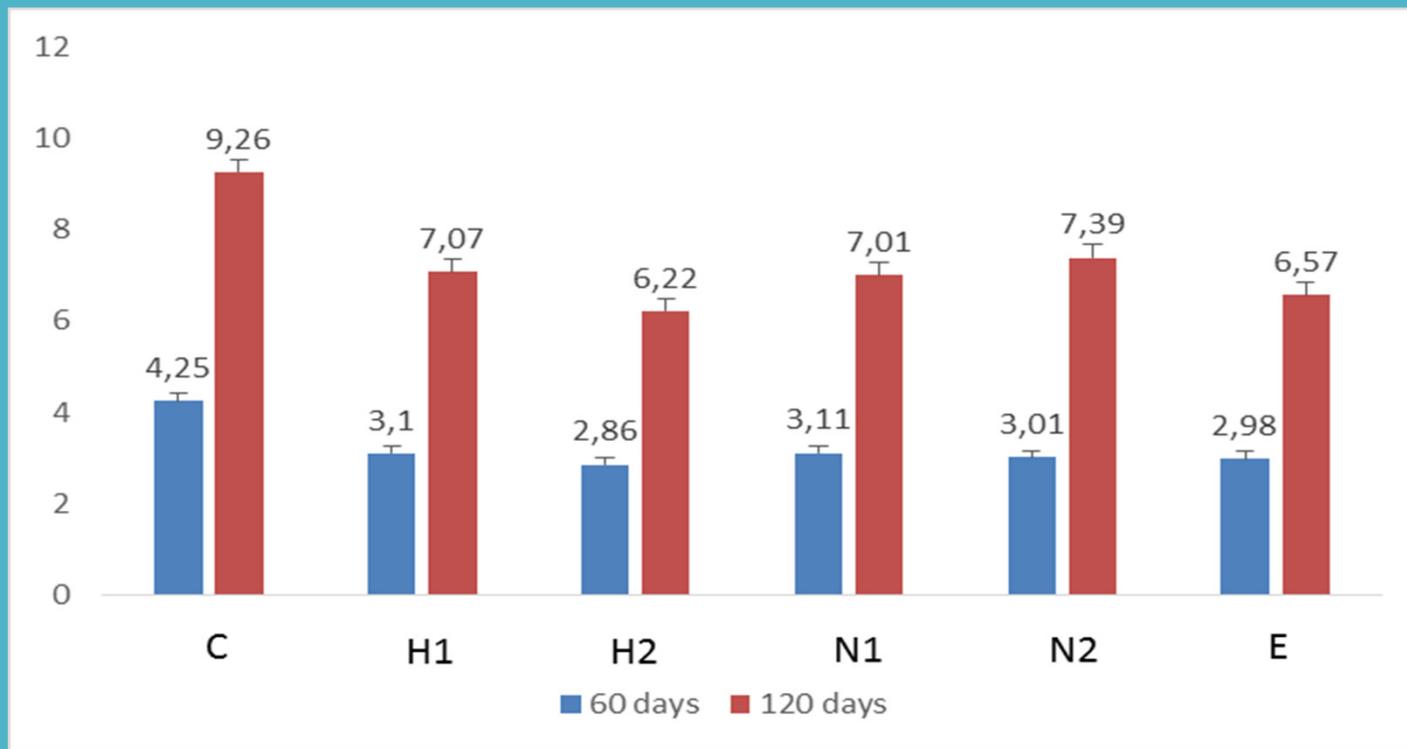
*Effect of dietary hesperidin or naringin or  $\alpha$ -tocopheryl acetate supplementation on egg yolk oxidative stability (ng MDA/g yolk) in laying hens after storage at room temperature  $\sim 20^{\circ}\text{C}$  (means  $\pm$  se) (higher levels of MDA indicate higher rates of lipid oxidation)*



C, 0 g/kg feed; H1, 0.75 g hesperidin per kg feed; H2, 1.5 hesperidin per kg feed; N1, 0.75 g naringin per kg feed; N2, 1.5 naringin per kg feed; E, 0.2 g  $\alpha$ -tocopheryl acetate per kg feed

## Results (3)

*Effect of dietary hesperidin or naringin or  $\alpha$ -tocopheryl acetate supplementation on egg yolk oxidative stability (ng MDA/g yolk) in laying hens after refrigerated storage at 4°C (means  $\pm$  se) (higher levels of MDA indicate higher rates of lipid oxidation)*



C, 0 g/kg feed; H1, 0.75 g hesperidin per kg feed; H2, 1.5 hesperidin per kg feed; N1, 0.75 g naringin per kg feed; N2, 1.5 naringin per kg feed; E, 0.2 g  $\alpha$ -tocopheryl acetate per kg feed

## Results (4)

*Effect of 62-d dietary hesperidin or naringin or  $\alpha$ -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; N1, 0.75 g naringin per kg feed; N2, 1.5 naringin per kg feed; E, 0.2 g  $\alpha$ -tocopheryl acetate per kg feed

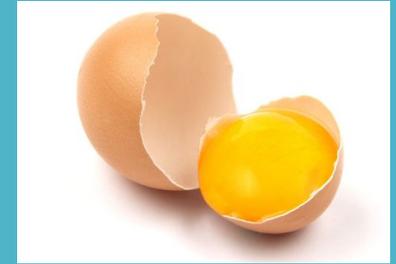
## Discussion (1)

- Yolk oxidative stability was improved even from the 4th day of the experiment, even at the low levels (0.75g/kg) ( $P < 0.001$ )
- This improvement was maintained after storage at room temperature (15-30 days) and refrigerator (60-120 days)
- Oxidation values of the bioflavonoids supplemented groups were similar to that of the  $\alpha$ -tocopheryl acetate supplemented group
- ✓ *Inclusion of hesperidin in laying hens' diets (1 g/kg) for 1 week significantly reduced yolk oxidation values (Goliomytis et al., 2014)*
- ✓ *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 (Lien et al., 2008; Ting et al., 2011)*

## Discussion (2)

- No effect of 62-day flavonoids dietary supplementation on yolk cholesterol level was observed
- ✓ No effect on yolk cholesterol level was observed after the incorporation of hesperidin into the hen diet for 28 days (Goliomytis et al., 2014)
- ✓ On the other hand, yolk cholesterol content appears to decrease 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) in previous studies (Lien et al., 2008; Ting et al., 2011)
- ❖ **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**

## Conclusions



- Egg yolk oxidative stability is improved after the dietary supplementation with the bioflavonoids (hesperidin and naringin) → egg quality is improved and shelf-life is increased
- Yolk cholesterol content was not different among the experimental groups
- However, further experimentation is warranted to elucidate the exact action of bioflavonoids in hens' metabolism

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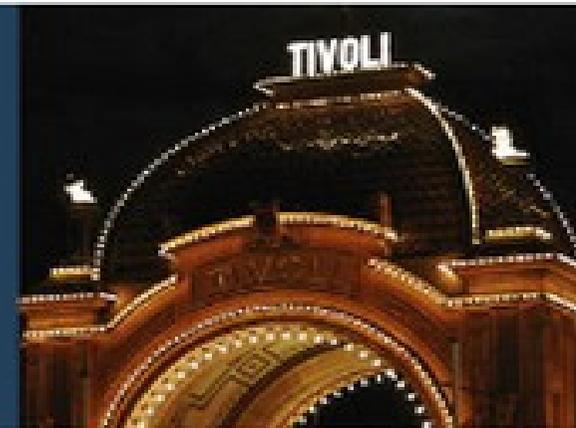


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*Thank you for your attention*

