Journal of Functional Foods 22 (2016) 454–462

Available online at www.sciencedirect.com

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journal homepage: www.elsevier.com/locate/jff

Alfalfa and flax sprouts supplementation enriches the content of bioactive compounds and lowers the cholesterol in hen egg

S. Mattioli, A. Dal Bosco, M. Martino, S. Ruggeri, O. Marconi, V. Sileoni, B. Falcinelli, C. Castellini, P. Benincasa *

Department of Agricultural, Food and Environmental Sciences, University of Perugia, Perugia 06121, Italy

ARTICLE INFO

Article history:
Received 28 November 2015
Received in revised form 29 January 2016
Accepted 4 February 2016
Available online 23 February 2016

Keywords:
Laying hens, eggs, sprouts
ω-3 fatty acid
Tocopherols
Phytochemicals

ABSTRACT

The effect of dietary supplementation with flax and alfalfa sprouts (40 g/d) on bioactive compounds and cholesterol contents of hen's egg was examined. Thirty White Leghorn hens, 26 weeks of age, were fed, for 66 days, three diets that included control (standard diet – C), standard diet + alfalfa sprouts (A), and standard diet + flax sprouts (F). Productive performance of hens was recorded daily. The cholesterol content of plasma and yolk, and the presence of bioactive compounds in the egg, were also analysed. Supplementation of flax and alfalfa sprouts reduced plasma and egg cholesterol probably due to the synergy between different compounds of the sprouts (polyunsaturated fatty acids - PUFAs, lignans, isoflavones and sterols). Eggs from A and F groups also had higher contents of n-3 PUFA, vitamins (α-tocopherol, α-, γ-tocotrienol, retinol), carotenes (β-carotene, lutein, zeaxanthin) and phytoestrogens (daidzein, equol, isolariciresinol) than eggs from the C group.

1. Introduction

No other single food of animal origin is eaten by so many people all over the world compared with the egg and none is served in such a variety of ways (Surai & Sparks, 2001). Therefore, the ability of the egg to be used as a functional food has been widely investigated (Stadelman, 1999) because the egg composition can be partly modified by changing the poultry feed (Lemahieu et al., 2015; Vaghefi, 2003). The majority of research has investigated the possibility of enriching eggs with fatty acids, vitamin E, selenium and lutein, but very few evaluated other bioactive phytochemicals (phytosterols, isoflavones, lignans, etc.) which are considered effective in human health (Finley, 2005; Webb & McCullough, 2005).

Phytochemicals are present in plant seeds and their contents are known to increase during germination in most plant species, from legumes, to oilseeds, to cereals (Alvarez-Jubete, Wijngaard, Arendt, & Gallagher, 2010; Baenas, Moreno, & Garcìa-Viguera, 2012; Benincasa et al., 2015; Marton, Mándoki, Csapo-Kiss, & Csapó, 2010). In fact, sprouts (i.e., the young seedlings obtained from seed germination) are becoming more and more popular in western countries as healthy foods, for their positive effects on the prevention of cardiovascular diseases

* *Corresponding author.* Department of Agricultural, Food and Environmental Sciences, University of Perugia, Perugia 06121, Italy. Tel.: +39 075 585 6325; fax: +39 075 585 6344.
E-mail address: paolo.benincasa@unipg.it (P. Benincasa).

Chemical compounds: Daidzein (PubChem CID: 5281708); Equol (PubChem CID: 91469); Phytosterols (PubChem CID: 12303662); Cholesterol (PubChem CID: 5997); Alpha-linolenic acid (PubChem CID: 5280934); Tocopherols (PubChem CID: 14986); Tocotrienols (PubChem CID: 9929901); Zeaxanthin (PubChem CID: 5280899); Lutein (PubChem CID: 5281243); Beta-carotene (PubChem CID: 5280489).

http://dx.doi.org/10.1016/j.jff.2016.02.007

and cancer (Ma et al., 2014). In particular, flax sprouts have been found to contain high levels of water-soluble proteins and free amino acids, free fatty acids and glycolipid fractions, lysophosphatidylcholine, and phosphatidic acid (Narina, Hamama, & Bhardwaj, 2012; Wanasundara, Shahidi, & Brosnan, 1999; Wanasundara, Wanasundara, & Shahidi, 1999). Alfalfa sprouts contain high amounts of vitamins A and C, coumestrol, liquiritigenin, isoliquiritigenin, loliolide, and saponins (Hong et al., 2011; Oleszek, 1998; Plaza, De Ancos, & Cano, 2003).

On the other hand, some public concern derives from the risk of bacterial contamination (e.g. *Escherichia coli*, *Salmonella enterica*, *Vibrio Cholerae*), because sprouts are normally home-made and used as components of salads, with no thermal or other sanitation treatment (Taormina, Beuchat, & Slutsker, 1999).

The use of sprouts in animal feeding could represent an alternative to transfer bioactive compounds from sprouts to livestock products and in turn to humans. This would be an attractive way to improve the quality and safety of food destined for human consumption together with animal health. However, very few studies have evaluated the possibility of transferring bioactive compounds from the sprouts to animal products (Dal Bosco et al., 2015), and none to hen's egg. Thus, the aim of the present study was to evaluate the effect of dietary supplementation of fresh alfalfa and flax sprouts on the bioactive compounds (phytosterols, phytoestrogens, tocopherols, carotenes, n-3 fatty acids) and the cholesterol contents of hen's egg.

2. Materials and methods

All used chemicals and reagents were at least of analytical grade and purchased from Sigma-Aldrich (Bornem, Belgium), unless otherwise specified.

2.1. Production of alfalfa and flax sprouts

Alfalfa (*Medicago sativa L.*) and flax (*Linum usitatissimum L.*) seeds were germinated on a substrate consisting of moistened tissue paper lying on a layer of silica sand sterilised at 105 °C in aluminium trays (22 cm × 30 cm for alfalfa and 30 cm × 36 cm for flax). In each tray, the sand (600 g for the alfalfa and 1 kg for the flax) was distributed to create a uniform layer on the bottom of the tray and moistened with demineralised water. The trays were placed in a temperature-controlled room at 20 °C in the dark and kept in these conditions for three days. Water was added periodically to compensate for sand water loss due to evaporation. In contrast to the usual sprouting procedure used for alfalfa in which sand water content is restored once a day, flaxseed requires several separate additions of water, as the seeds tend to produce a glue-like mucilaginous exudate in the presence of high water content that would hamper seedling development. For each species, the sprouts obtained on the third day from different trays were combined to prevent a possible tray effect and stored at 4 °C in plastic bags until use (i.e., within three days).

2.2. Animals and diets

The experimental protocol was devised according to the Italian directives (Gazzetta Ufficiale, 1992) on animal welfare and the research was carried out at the experimental farm of the Department of Agricultural, Food and Environmental Science of the University of Perugia (Italy) from November 2013 to February 2014.

Table 1 – Mean ingredients (g kg^{-1}) of diet and nutrient composition (g kg^{-1}) of diet and sprouts.

	Feed	Alfalfa	Flax
Ingredients			
Maize	450		
Extruded soybean flakes	200		
Maize gluten feed	160		
Sunflower meal	88		
Alfalfa meal	30		
Vitamin mineral premix*	10		
Calcium carbonate	50		
Dicalcium phosphate	5		
Sodium bicarbonate	5		
Salt	2		
Nutrient composition			
Water	11.0	87.6	87.5
Crude protein	178	68	61
Ether extract	53	5.2	6.3
Crude fibre	56	30	36
Ash	111	20	21

* Provided per kilogram of diet: vitamin A, 12,500 IU; cholecalciferol, 3,000 IU; DL-alpha-tocopheryl acetate, 60 mg; Vitamin B_1, 2 mg; Vitamin B_2, 6 mg; Vitamin B_6, 4 mg; pantothenic acid, 8 mg; PP 30 mg; folic acid, 0.50 mg; vitamin B_{12}, 0.02 mg; vitamin K, 2 mg; choline, 750 mg; Fe, 35 mg; Zn, 42 mg; I, 0.5 mg; Co, 0.5 mg.

Thirty White Leghorn hens, 30 wk. of age at the start of experiment, were randomly assigned to one of the following conditions:

- Standard diet (C);
- Standard diet + 40 g/d of alfalfa sprouts (A);
- Standard diet + 40 g/d of flax sprouts (F).

The hens of each group were kept in indoor pens under standard housing conditions with an artificial photoperiod of 16 h per day of light was applied. The building was under a controlled ventilation regime (10 m^3/hen/h): the temperature ranged from 23 to 27 °C, and the relative humidity ranged from 50 to 80%.

Standard feed and water were provided with manual bell feeders and automatic drinkers. Feed and water were provided *ad libitum* (Table 1) to all the groups and the daily residues were weighed for evaluation of voluntary feed intake. Fresh sprouts were placed daily near the feeders.

The overall experimental period lasted 66 days. Egg deposition was recorded daily; in particular the deposition rate was evaluated the wk. before the experiment started (baseline day 0) and at the end of sprout supplementation.

2.3. Eggs and blood sampling

For each dietary treatment, 10 pools of 10 egg yolks/per group were collected at day 0 and day 66 stored at 5 °C until the analyses (maximum 2 days after) that were performed in the laboratory of the department. The blood was sampled at 0 and 66 day from the brachial vein of 10 hens per group, in heparinised vacutainers and centrifuged at 1500 × g for 15 min at +4 °C, to measure the plasma cholesterol concentration.