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Effect of free-range feeding on $n-3$ fatty acid and α-tocopherol content and oxidative stability of eggs

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Abstract

This study was carried out to compare the fatty acid and α-tocopherol composition and the susceptibility to oxidation of egg yolks from hens fed grass and a commercial mixed diet under free-range conditions or in cages with only the commercial diet. Grass had a relative high proportion of α-linolenic acid (534 g kg^{-1} total fatty acids) and α-tocopherol (162.3 mg kg^{-1} DM). Eggs from hens fed under free-range conditions had a higher concentration of total $(n-3)$ fatty acids than eggs from hens fed the commercial diet ($P<0.05$). Eggs from layers on free-range had a higher concentration of α-tocopherol than those of hens maintained in cages and fed the commercial diet ($P<0.01$). No differences in initial values or rate of oxidation were observed between treatments. This research suggests that some constituents of grass may be of interest for the production of eggs rich in $(n-3)$ fatty acids, without adverse oxidative effects.

Keywords: Feeding; Free-range; Yolk; α-Tocopherol; Oxidation

1. Introduction

Intensive non-ruminant animal production has resulted in a change in feed composition with a partial or total substitution of grass, rich in α-linolenic acid, for feed constituents with a relative high content of linoleic, oleic or saturated fatty acids (Crawford et al., 1970; National Research Council, 1994). This has resulted in a

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decrease of α-linolenic and other $(n-3)$ fatty acids in animal lipids (Crawford et al., 1970, 1984).

In recent years, the lipid composition of chicken egg has been a primary area of consumer concern due to the relationship of specific dietary lipids with the development of coronary heart disease and some forms of cancer (Simopoulos and Salem, 1992). As a result, feeding strategies are being incorporated to increase the $(n-3)$ fatty acid content of chicken eggs (Cherian et al., 1996a,b). However, with increasing polyunsaturated fatty acids (PUFA) content of poultry diets, there is a concomitant increase in the susceptibility to oxidative deterioration of eggs, leading to losses in quality characteristics and nutritional value, lower consumer acceptability and deleterious biological effects (Addis and Park, 1989). The susceptibility of animal lipid to oxidation depends also on a number of additional factors, including the concentration of prooxidants and antioxidants (Monahan et al., 1992). The effect of dietary α-tocopheryl acetate supplementation on enhancing lipid stability in animal food products and particularly in egg yolk has been extensively reported (Aymond and van Elswyk, 1995; Cherian et al., 1996a; Lopez-Bote et al., 1997).

Diets of laying hens raised on free-range conditions include grass, which has not only a relative high level of essential $(n-3)$ fatty acids, but also a relative high level of tocopherols and some other non-saponifiable lipid components that may be of importance for the quality characteristics of eggs (Brown, 1953; Lynch, 1991; Mutetikka and Mahan, 1993; Tramontano et al., 1993).

Although it has been reported that forage can affect the nutrient status of domestic animals (Mutetikka and Mahan, 1993), no study, to our knowledge, has been conducted comparing the resulting egg fatty acid content, tocopherol level and susceptibility to oxidation when hens are fed under free-range conditions or with formulated mixed feeds in cage environment.

2. Material and methods

2.1. Animals and diets

Twenty-five 13-month-old single Comb White leghorn laying hens were housed in two double-deck cage batteries with one bird per cage (0.31×0.40 m). The hens were given free access to a conventional diet for layers (Table 1). Another group of 25 birds was fed in free-range conditions according to the European Union regulations for the specific term of free-range (Thear, 1990) with grass fully available. The land used was near the house and 0.5 ha without fences. The approximate mean distance of birds from the house was 25 m. Several trees provided protection from wind, rain and sun. Two suspended plastic drinkers filled manually every day were used. Natural grassland included several species of grass (mainly Italian raygrass, *Lolium perenne*), legumes and herbs. These birds were given 50 g of mixed feed (same feed as in the other group) per hen in the house daily in manually filled tube feeders at fairly low level to prevent wastage, and available on an ad libitum basis. Samples of grass were taken and analyzed weekly from random locations by cutting the grass on the same area where the hens

Table 1
Composition and determined analysis of experimental mixed feed of hens fed in cages and on free-range and determined analysis of grass

	Mixed Feed	Grass
Ingredients (g kg^{-1})		
Corn	530.0	
Wheat	50.0	
Full fat soybean	47.0	
Soybean meal (440 g kg^{-1} CP)	196.4	
Lard	21.0	
Meat meal (520 g kg^{-1} CP)	50.0	
NaCl	3.0	
Calcium carbonate	76.1	
Dicalcium phosphate	9.0	
Premix[a]	17.7	
Calculated ME (MJ kg^{-1})	12.13	
Dry matter (DM) (g kg^{-1})	893.8	263.5
Crude protein (g kg^{-1} DM)	180.6	137.2
Fat (g kg^{-1} DM)	59.21	62.6
Crude fiber (g kg^{-1} DM)	27.9	222.2
Ca	37	
P	4.2	
α-Tocopherol (mg kg^{-1} DM)	12.24	162.31
Fatty acids (g kg^{-1} total fatty acids)		
C16:0	183.5	185.2
C16:1 ($n-7$)	11.9	4.2
C18:0	101.9	24.1
C18:1 ($n-9$)	302.8	111.3
C18:2 ($n-6$)	373.6	140.6
C18:3 ($n-3$)	26.2	534.6

[a]Provided per kg of diet: vitamin A, 8800 IU; vitamin D, 2200 IU; vitamin E, 6.0 IU; vitamin K, 2.2 mg; thiamine, 1.1 mg; riboflavin, 4.4 mg; pantothenic acid, 12 mg; niacin, 22 mg; choline, 500 mg; vitamin B_{12}, 0.013 mg; biotin, 0.055 mg; manganese, 69 mg; zinc, 55 mg; iron, 26 mg; copper, 4.4 mg; iodine, 1.1 g; selenium, 0.1 mg.

remained. A 20-cm square ring was thrown three times and grass was cut by means of a garden scissor. Chemical composition and fatty acid composition of experimental diets are shown in Table 1. Chemical analysis of feed was carried out as previously described (Lopez-Bote et al., 1997).

Throughout the experiments, the hens were handled according to the principles for the care of animals in experimentation (National Research Council, 1985).

2.2. Sample collection and chemical analysis

After 28 days on experiment, one egg from each of the hens housed in cages was collected. An equal number of eggs were taken from nesting houses of free-range layers. The hens were marked with leg ringing and trap nest were used. The eggs from cage birds were collected daily, while those from free-range layers were collected every 2 h

from 0900 to 1700. Eggs were stored under refrigeration and, within 10 days following the day of collection, were cracked and yolks were separated and analyzed.

Lipids from yolks were extracted by the procedure described by Bligh and Dyer (1959) and analyzed as previously described (Lopez-Bote et al., 1997).

The susceptibility of egg yolk to iron-induced lipid oxidation was determined by a modification of the method of Kornbrust and Mavis (1980) in which $FeSO_4$ was used as the catalyst of lipid oxidation. Homogenates (approximately 1 g l^{-1} buffer) were incubated at 37°C in 0.04 mol l^{-1} tris-maleate buffer (pH 7.4) with 0.001 mol l^{-1} $FeSO_4$ in a total volume of 10 ml. At fixed time intervals, aliquots were removed for measurement of thiobarbituric acid reactive substances (TBARS).

For the determination of α-tocopherol, 1 g sample was homogenized in 10 ml 0.054 M dibasic sodium phosphate buffer adjusted to pH = 7.0 with HCl. After mixing with absolute ethanol and hexane, the upper layer containing tocopherol was evaporated and redissolved in ethanol prior to analysis by reverse phase HPLC (Hewlett Packard 1050, with a UWD, HPIB 10 detector, RP-18 endcapped column, Waldbronn, Germany)(Rey et al., 1996). The mobile phase was methanol:water (97:3).

2.3. Statistical analysis

An individual egg was the experimental unit for analysis of all data. Response data were evaluated by the GLM procedure (SAS Institute, 1988).

3. Results and discussion

The chemical and major fatty acid composition of diets is shown in Table 1. Since the mixed diet available to both groups was identical, differences in egg yolk composition and characteristics should be attributed mainly to the grass intake. Grass had a relative high proportion of α-linolenic acid (C18:3, $n-3$) (534 g kg^{-1})(Table 1). Rice et al. (1981), Burgstaller and Jatsch (1991) and Hakkarainen and Pehrson (1987) also reported a relative high proportion of C18:3 ($n-3$) in samples from pasture. The α-tocopherol content of the grass was 162.3 mg kg^{-1} DM (Table 1). The concentration of α-tocopherol in pastures has been quantified by several authors with contradictory results. While Brown (1953) and Mutetikka and Mahan (1993) found a decline in α-tocopherol content as plant mature, Tramontano et al. (1993) reported an increase in α-tocopherol concentration with plant age. In general, reported values of α-tocopherol content are relatively high when compared to common recommended values for feeding laying hens (National Research Council, 1994) and range from 70 to 200 mg kg^{-1} (expressed on a dry matter basis) (Brown, 1953; Lynch, 1991; Mutetikka and Mahan, 1993; Tramontano et al., 1993; Rey et al., 1997).

The fatty acid composition of yolk lipids, as affected by experimental diets, is shown in Table 2. The major fatty acids were oleic (C18:1, $n-9$), palmitic (C16:0) and linoleic (C18:2, $n-6$). These data are similar to those reported in the literature (Guardiola et al., 1994; Cherian et al., 1996a,b). Eggs from hens fed on free-range had higher concentration of C18:3 ($n-3$) ($P = 0.065$), eicosapentaenoic (C20:5, $n-3$)

Table 2
Fatty acid composition (g kg^{-1} total fatty acids) of the yolk fat from hens fed mixed feed in cages (MF) or mixed feeds and grass under free-range conditions (FR) ($n = 22$)

	MF	FR	SEM	$P > F$[a]
C14:0	0.32	0.39	0.006	0.003
C14:1	0.04	0.06	0.004	NS
C15:0	0.09	0.10	0.012	NS
C16:0	24.04	27.00	0.225	0.001
C16:1 ($n-9$)	0.70	0.26	0.042	0.002
C16:1 ($n-7$)	2.27	2.65	0.085	NS
C18:0	13.11	14.05	0.692	NS
C18:1 ($n-9$)	35.98	36.91	0.794	NS
C18:1 ($n-7$)	0.08	0.10	0.003	0.071
C18:2 ($n-6$)	18.70	12.00	0.488	0.001
C19:0	0.11	0.11	0.003	NS
C18:3 ($n-3$)	0.39	0.99	0.070	0.065
C20.0	0.02	0.04	0.001	0.001
C20:1 ($n-9$)	0.25	0.26	0.006	NS
C20:3 ($n-9$)	0.19	0.22	0.022	NS
C20:4 ($n-6$)	2.11	2.01	0.041	NS
C20:5 ($n-3$)	0.02	0.15	0.021	0.044
C22:1 ($n-9$)	0.01	0.11	0.029	NS
C23:0	0.02	0.01	0.005	NS
C22:4 ($n-6$)	0.19	0.28	0.005	0.001
C22:5 ($n-6$)	0.58	0.43	0.024	0.028
C22:5 ($n-3$)	0.13	0.31	0.029	0.032
C22:6 ($n-3$)	0.62	1.57	0.063	0.001
Σ ($n-3$)	1.16	3.02	0.162	0.001
Σ ($n-6$)	21.59	14.72	0.491	0.001
Σ ($n-6$)/Σ ($n-3$)	18.73	5.21	0.449	0.001
Σ sat	37.71	41.68	0.763	0.072
Σ mono	39.34	40.35	0.836	NS
UI[b]	95.08	91.01	1.497	NS

[a] NS = not significant ($P > 0.1$).
[b] UI (unsaturation index) = average number of double bonds per fatty acid residue × 100.

($P = 0.044$), docosapentaenoic (C22:5, $n-3$) ($P = 0.032$) and docosahexaenoic (C22:6, $n-3$) ($P = 0.001$) acids than eggs from hens fed the conventional diet alone. The concentration of ($n-3$) fatty acid was almost threefold higher in eggs from hens fed on free-range ($P < 0.001$). On the other hand, the concentration of ($n-6$) fatty acids was higher in eggs from hens fed the commercial diet in confinement, reflecting the higher concentration of C18:2 ($n-6$) in the feed. Guardiola et al. (1994) reported higher ($n-6$)/($n-3$) ratio in egg lipids from large scale farms, fed with higher proportion of commercial feed.

Some reports indicate that ($n-3$) fatty acids are particularly susceptible to lipid oxidation, and even small differences in the concentration of these fatty acids may be critically important in the development of oxidation. Hu et al. (1989) conducted an experiment in which they compared the susceptibility of tissue from rats fed diets high

in $(n-3)$ or $(n-6)$ PUFA to in vitro lipid peroxidation and observed higher levels of TBARS in tissues of those receiving higher levels of $(n-3)$ fatty acids. This is consistent with other investigators who suggested enhanced susceptibility to lipid peroxidation of $(n-3)$ fatty acids either as pure lipid or in tissues of rats fed fish oil compared to rats fed corn oil (Hammer and Wills, 1978).

According to the results of the concentration of $(n-3)$ fatty acids, susceptibility to lipid peroxidation between egg yolk from the two experimental groups would be expected to be higher in those from hens fed on free-range conditions.

The concentration of α-tocopherol in egg yolk is shown in Table 3. Eggs from layers on free-range had higher concentration of α-tocopherol than those of hens maintained in cages with mixed diets ($P < 0.01$). Mutetikka and Mahan (1993) reported that feeding gilts on pasture provided a dietary source of vitamin E that resulted in a concentration of α-tocopherol in milk and serum higher than in gilts fed mixed diets supplemented with α-tocopheryl acetate (22 mg kg^{-1} feed). A higher muscle and microsome extracts α-tocopherol concentration in pigs fed a diet containing grass than in those fed commercial feeds has been also reported (Rey et al., 1997).

Although grass intake was not measured and species selection by hens could not be determined, visual appraisal of the pasture lot suggested that the daily quantity of grass consumed was high throughout the entire experimental period.

In order to evaluate the specific local effect of fatty acids or α-tocopheryl acetate upon the yolk lipids to oxidation, analysis of stability to $FeSO_4$ was carried out. The rate of iron-induced lipid peroxidation is presented in Table 3. No differences in initial values or rate of oxidation were observed between treatments.

Several reports indicate that dietary administration of supplemental levels of α-tocopheryl acetate improve the stability of animal fat (Monahan et al., 1992; Lopez-Bote et al., 1997). The lower oxidation than expected according to the fatty acid composition is noteworthy and may be attributed to the higher concentration of α-tocopherol in the yolk. Some other dietary constituents not quantified in this experiment might also play a protective role. A number of papers have been published in recent years in which the antioxidant effect of dietary natural antioxidants other than α-tocopherol were shown (Elmadfa, 1995; Lopez-Bote et al., 1998).

Table 3
Concentration of α-tocopherol in the yolk and iron-induced lipid peroxidation of yolk from hens fed mixed feeds in cages (MF) or mixed feed and grass under free-range conditions (FR) ($n = 22$)

	MF	FR	SEM	$P > F$[a]
α-Tocopherol ($\mu g\ g^{-1}$ yolk)	65.58	86.22	3.025	0.010
Incubation time (min)	(mmol malonaldehyde kg^{-1} yolk)			
0	0.65	0.49	0.225	NS
30	1.30	1.15	0.272	NS
60	1.38	1.22	0.264	NS
Increment	0.73	0.74	0.162	NS

[a] NS = not significant ($P > 0.05$).

This research suggests that some constituents of grass may be of interest for the production of eggs rich in $(n-3)$ fatty acids, without adverse oxidative effects.

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References

Addis, P.B., Park, S.W., 1989. Role of lipid oxidation products in atherosclerosis. In: Scanlan, R., Taylor, S.L. (Eds.), Food Toxicology. Marcel Dekker, New York, NY, pp. 297–330.

Aymond, W.N., van Elswyk, M.E., 1995. Yolk thiobarbituric acid reactive substances and $n-3$ fatty acids in response to whole and ground flaxseed. Poult. Sci. 74, 1388–1394.

Bligh, E.G., Dyer, W.J., 1959. A rapid method of total lipid extraction and purification. Can. J. Biochem. Physiol. 37, 911–917.

Brown, F., 1953. The tocopherol content of farm feedstuffs. J. Sci. Food Agric. 4, 161–165.

Burgstaller, G., Jatsch, C., 1991. Fattening of heavy pigs, up to 160 kg liveweight, for the production of long-life sausage based on home-produced forage: II. Influence of grass meal on fatty acid pattern of mesenteric fat and backfat. Wirtschaftseigene-Futter 37, 308–317.

Cherian, G., Wolfe, F.H., Sim, J.S., 1996a. Dietary oils with added tocopherols: effects on egg or tissue tocopherols, fatty acids and oxidative stability. Poult. Sci. 75, 423–431.

Cherian, G., Wolfe, F.H., Sim, J.S., 1996b. Feeding dietary oils with tocopherols: effect on internal qualities of eggs during storage. J. Food Sci. 61, 15–18.

Crawford, M.A., Gale, M.M., Woodford, M.H., 1970. Muscle and adipose tissue lipids of the wart hog (*phacochoerus aethiopicus*). Int. J. Biochem. 1, 654–658.

Crawford, M.A., Hare, W.R., Whitehouse, D.B., 1984. Nutrient partitioning in domesticated and nondomesticated animals. In: Wiseman, J. (Ed.), Fats in Animal Nutrition. Butterworth, London, pp. 471–479.

Elmadfa, I., 1995. Physiological importance of unsaponifiable components in dietary fat. Fett Wiss. Technol. 97, 85–90.

Guardiola, F., Codony, R., Rafecas, M., Boatella, J., Lopez, A., 1994. Fatty acid composition and nutritional value of fresh eggs from large- and small-scale farms. J. Food Comp. Anal. 7, 171–188.

Hakkarainen, J., Pehrson, B., 1987. Vitamin E and polyunsaturated fatty acids in Swedish feedstuffs for cattle. Acta Agric. Scand. 37, 341–346.

Hammer, C.T., Wills, E.D., 1978. The role of lipid components of the diet in the regulation of the fatty acid composition of the rat liver endoplasmic reticulum and lipid peroxidation. Biochem. J. 174, 585–593.

Hu, M.L., Frankel, E.N., Leibovitz, B.E., Tappel, A.L., 1989. Effect of dietary lipid and vitamin E on in vitro lipid peroxidation in rat liver and kidney homogenates. J. Nutr. 119, 1574–1582.

Kornbrust, D.J., Mavis, R.D., 1980. Relative susceptibility of microsomes from lung, heart, liver, kidney, brain and testes to lipid peroxidation: correlation with vitamin E content. Lipids 15, 315–322.

Lopez-Bote, C.J., Rey, A., Sanz, M., Gray, J.I., Buckley, J.D., 1997. Dietary vegetable oils and α-tocopherol reduce lipid oxidation in rabbit muscle. J. Nutr. 127, 1176–1182.

Lopez-Bote, C.J., Gray, J.I., Gomaa, E., Flegal, C.J., 1998. Effect of dietary administration of oil extracts from rosemary and sage on lipid oxidation in broiler meat. Br. Poult. Sci. (in press).

Lynch, G.L., 1991. Natural occurrence and content of vitamin E in feedstuffs. In: Coelho, M.B. (Ed.), Vitamin E in Animal Nutrition and Management. BASF, Parsippany, NJ, pp. 43–48.

Monahan, F.J., Buckley, D.J., Morrissey, P.A., Lynch, P.B., Gray, J.I., 1992. Influence of dietary fat and α-tocopherol supplementation on lipid oxidation in pork. Meat Sci. 31, 229–241.

Mutetikka, D.B., Mahan, D.C., 1993. Effect of pasture, confinement, and diet fortification with vitamin E and selenium on reproducing gilts and their progeny. J. Anim. Sci. 71, 3211–3218.

National Research Council, 1985. Guide for the Care and Use of Laboratory Animals. Publication no. 85-23, NIH, Washington, DC.

National Research Council, 1994. Nutrient Requirements of Poultry. National Academy Press, Washington, DC.

Rey, A.I., Lopez-Bote, C.J., Soares, M., Isabel, B., 1996. Determination of α-tocopherol in pork with high intramuscular fat content. Grasas Aceites 47, 331–334.

Rey, A.I., Lopez-Bote, C.J., Sanz Arias, R., 1997. Effect of extensive feeding on α-tocopherol concentration and oxidative stability of muscle microsomes from Iberian pigs. Anim. Sci. 65, 515–520.

Rice, D.A., Blanchflower, W.J., McMurray, C.H., 1981. Reproduction of nutritional degenerative myopathy in the post ruminant calf. Vet. Rec. 109, 161–162.

SAS Institute, 1988. SAS User's Guide: Statistics. SAS Institute, Cary, NC.

Simopoulos, A.P., Salem, N. Jr., 1992. Egg yolk as a source of long-chain polyunsaturated fatty acids in infant feeding. Am. J. Clin. Nutr. 55, 411–414.

Thear, K., 1990. Free-Range Poultry. Farming Press, Ipswich, UK.

Tramontano, W.A., Ganci, D., Pennino, M., Dierenfeld, E.S., 1993. Distribution of α-tocopherol in early foliage samples in several forage crops. Phytochemical 34, 389–390.