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Improving the fatty acid profile in egg yolk through the use of hempseed (*Cannabis sativa*), ginger (*Zingiber officinale*), and turmeric (*Curcuma longa*) in the diet of Hy-Line White Leghorns

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Abstract. A study was performed to examine the outcome of utilizing hempseed, ginger, and turmeric in the diet of Hy-Line White Leghorn on the fatty acid profile of egg yolk. Four experimental rations were offered to 60 laying hens: control (standard diet); 25 % hempseed (T1); 25 % hempseed and 2 % turmeric (T2); 25 % hempseed and 2 % ginger (T3). Thirty eggs per group were analysed on day 0, 15, and 30 after storage at room temperature. Individual and total fatty acids decreased significantly ($P < 0.05$) in the experimental groups by day 0, 15, and 30 compared to the control. By day 0, 15, and 30, total monounsaturated fatty acids, palmitoleic acid, and oleic acid decreased significantly ($P < 0.05$) in T3. In fresh eggs, polyunsaturated fatty acids (PUFAs) increased significantly in T2 and T3 and were lower in the control and T1 by day 15 and 30. The same trend was also found in linoleic, eicosadienoic, and arachidonic acid. Linolenic acid increased significantly ($P < 0.05$) in T2 and T3 by day 15 and 30. However, eicosatrienoic acid, eicosapentaenoic acid, and docosahexaenoic acid increased significantly ($P < 0.05$) in all treated groups compared to the control by day 15 and 30. By day 15 and 30, total ω-3 increased significantly in treated groups. In contrast to ω-3, ω-6 increased significantly in T2 and T3 by day 15 and 30, while their ratio decreased significantly in treated groups. From the results of the present study, it was concluded that the addition of hempseed at the level of 25 % combined with 2 % turmeric and ginger in the diet of Hy-Line White Leghorn layers improved the fatty acid profile of n-3 PUFA, ω-3, and ω-6 in egg yolk.

1 Introduction

The function of essential fatty acids (linoleic and alpha-linolenic acid) and their metabolites in human and animal health is a subject of scientific attention today (Shahid et al., 2015). Because of inadequate ω-3 polyunsaturated fatty acid intake in the human diet, research work has been focused on producing animal food products enriched with a healthy fatty acid profile (Cherian, 2002; Kang, 2008). Among the various animal food stuffs evaluated for ω-3 fatty acid enhancement, hen egg has been reported as the easiest and the most successful means of adding ω-3 fatty acid to the human diet (Leskanich and Noble, 1997; Baucells et al., 2000; Cherian et al., 2007; Neijat et al., 2015). Different materials originating from plants, such as flaxseed, chia, and hempseed (Cherian and Sim, 1991; Ayerza and Coates, 1999; Gakhar et al., 2012; Neijat et al., 2015), and various full-fat seeds (Mazalli et al., 2004) are extremely successful in improving the fatty acid profile in eggs.

Hemp (*Cannabis sativa* L.) is an annual herb and belongs to the family Cannabinaceae (Turner et al., 1979). Hempseed contains approximately 25 % protein, 33 to 35 %

oil, and 34 % carbohydrates, as well as a wide range of vitamins and minerals (Callaway, 2004; House et al., 2010). Hempseed oil contains up to 80 % polyunsaturated fatty acids, in which linoleic acid and alpha-linolenic acid are as high as 60 and 19 % respectively (Parker et al., 2003). Hen egg contains more than 11 % fat, of which 33–35 % is in the yolk, and a large amount of it is unsaturated fats. However, ω-3-enriched eggs are prone to fatty acid lipid peroxidation by the process of oxidation leading to oxidative spoilage of egg yolk lipids, thus reducing the quality of the egg's features. This is caused during storage, leading to deteriorating egg organoleptic properties (Ahn et al., 1995). The egg's dual band is sensitive to oxidation and spoilage, which cause peroxidation, a change in odour and texture, the loss of nutrients, and the creation of toxic mixtures (Yang and Chen, 2001). Antioxidants reduce the effect of these radicals, avoiding spoilage (Surai et al., 2003).

Antioxidants are usually included in the laying hen's diet to reduce lipid peroxidation and to increase consumer interest in using ω-3-enriched eggs (Qi and Sim, 1998; Galobart et al., 2001). Natural and synthetic antioxidants are used to prevent lipid oxidation (Yamamoto and Niki, 1990). Synthetic antioxidants like butylated hydroxy toluene (BHT) and butylated hydroxyl anisole (BHA) have been used to prevent lipid peroxidation in foods (Imaida et al., 1983; Okada et al., 1990). Natural antioxidants include various plants and their parts (Cuppett and Hall, 1998; Nakatani, 2000; Wei and Shibamoto, 2007). The majority of herbs having antioxidant properties belong to the family Labiatae (plants of the mint family); these are rosemary, thyme, and oregano (Cuppett and Hall, 1998). Other antioxidant herbs belong to the family Zingiberaceae (turmeric and ginger) and Umbelliferae; in addition, plants rich in flavonoids and anthocyans have antioxidative characteristics (Nakatani, 2000; Wei and Shibamoto, 2007).

Turmeric (*Curcuma longa* L.) is used in cooking as a spicing and colouring agent. It is also used for the treatment of injuries, swellings, and sprains (Ammon and Wahl, 1991). Turmeric contains proteins (6.3 %), fats (5.1 %), minerals (3.5 %), carbohydrates (69.4 %), and moisture (13.1 %). The essential oil (5.8 %) contains alpha-phellandrene (1 %), sabinene (0.6 %), cineol (1 %), borneol (0.5 %), zingiberene (25 %), and sesquiterpenes (53 %) (Kapoor, 1990). Curcumin (diferuloylmethane) is responsible for the yellow colour and is comprised of curcumin I (94 %), curcumin II (6 %), and curcumin III (0.3 %) (Ruby et al., 1995). Curcumin also has antioxidant activity (Khan et al., 2012a). It is said to destroy oxygen free radicals (Subramanian et al., 1994; Ruby et al., 1995; Khan et al., 2012a).

Ginger (*Zingiber officinale*) has been used for cooking and medicine for numerous years (Chrubasik et al., 2005). In China, ginger has been used as a flavouring substance and against nausea for many decades. Ginger is now grown in Africa, Asia, and many other countries and is used internationally as treatment for nausea. This herb contains several agents such as gingerol, gingerdiol, and gingerdione that have powerful antioxidant action (Khan et al., 2012b). Gingerol has analgesic, antipyretic, sedative, and antibacterial effects in animals (Dieumou et al., 2009; Khan et al., 2012b). Fresh ginger has 80.9 % moisture, 2.3 % protein, 0.9 % fat, 1.2 % minerals, 2.4 % fiber, and 12.3 % carbohydrates. The chief minerals in ginger are iron, calcium, and phosphorus. It also contains numerous vitamins such as thiamine, riboflavin, niacin, and vitamin C. The composition fluctuates with the variety, agronomic circumstances, type, treatment method, drying method, and storage environment (Govindarajan, 1982).

To the best of our knowledge, a combination of hempseed, ginger, and turmeric have not been used for preserving fatty acid in eggs of laying hens. The present study was conducted to explore the effect of the addition of hempseed, ginger, and turmeric in a Hy-Line diet on the fatty acid profile in fresh and stored eggs.

2 Materials and methods

The study was approved by the Ethics Committee on the Welfare of Animals, Faculty of Animal Husbandry & Veterinary Sciences, The University of Agriculture, Peshawar, Pakistan.

2.1 Bird husbandry and experimental diets

Sixty hens (Hy-Line White Leghorns) aged 30 weeks were divided into four groups. Each group was replicated three times with five birds per replicate. The basic diet composition of the laying hens is given in Table 1. The diets consisted of a basal diet (control), 25 % hempseed (T1), 25 % hempseed (*Cannabis sativa*) and 2 % *Curcuma longa* (T2), and 25 % hempseed and 2 % *Zingiber officinale* (T3). The feed was offered at the rate of 130 g day^{-1}. Birds were reared in battery cages. Light was provided for 16 h day^{-1} throughout the experiment. The experiment lasted for 5 weeks. Nine eggs per group (three eggs per replicate) were collected at random and analysed at 0, 15, and 30 days of storage (room temperature). Eggs were broken and the egg yolk was separated and stored at −20 °C until analysis.

2.2 Determination of fatty acid

Fatty acid methyl esters (FAME) were measured according to the method of Shahid et al. (2015). Briefly, 50 mg egg yolk was mixed with 1.5 mL of methanolic sodium hydroxide and boiled at 100 °C for 5 min. After cooling, 2.5 mL boron trifluoride (14 %) was added into the tubes to collect the residual lipids (30 min at 80 °C) Fatty acid esters were separated in 1 mL hexane. Five millilitres of saturated salt (400 g of NaCl L^{-1}) was added, and samples were vortexed for 2 min, followed by centrifugation at 800 × g for 5 min. The top hexane layer was again transferred into a new gas

Table 1. Ingredient composition (%) and calculated nutritional composition of layer ration.

Ingredients	Control	T1	T2	T3
Hempseed	0	25	25	25
Curcuma longa	0	0	2	2
Zingiber officinale	0	0	2	2
Corn	51.25	41.25	41.25	41.25
Canola meal	6	5	5	5
Soybean meal	8	6	6	6
Fish meal	4	4	4	4
Sunflower meal	10	0	0	0
Corn gluten meal	4	0	0	0
Soya oil	4	0	0	0
Wheat bran	0	6	6	6
Marble chips	7	7	7	7
Dicalcium phosphate	2	2	2	2
Molasses	3	3	3	3
Lysine	0.2	0.2	0.2	0.2
Methionine	0.05	0.05	0.05	0.05
Salt	0.15	0.15	0.15	0.15
Soda	0.05	0.05	0.05	0.05
Coccidiostat	0.05	0.05	0.05	0.05
Zinc bacitracin	0.05	0.05	0.05	0.05
Vitamin mineral premix	0.2	0.2	0.2	0.2
Calculated nutritional composition				
Dry matter (%)	88.86	88.38	88.42	88.46
Metabolizable energy (Kcal kg^{-1})	2916	2911	2913	2912
Crude protein (%)	17.05	17.10	17.09	17.07
Ether extract (%)	10.03	10.02	10.03	10.02
Fiber (%)	8.94	8.96	8.95	8.97
Ash (%)	10.36	10.37	10.40	10.38
Calcium (%)	3.52	3.54	3.53	3.52
Available phosphorus (%)	0.59	0.59	0.59	0.59
Linoleic acid (%)	1.39	8.56	8.57	8.58
Lysine (%)	0.85	0.89	0.87	0.86
Methionine (%)	0.40	0.42	0.41	0.40
Cysteine (%)	0.28	0.29	0.28	0.28
Methionine and cysteine (%)	0.73	0.72	0.71	0.72
Salt (%)	0.41	0.40	0.39	0.38
Threonine (%)	0.59	0.58	0.59	0.57
Tryptophan (%)	0.18	0.17	0.17	0.18

T1: hempseed; T2: 25 % hempseed and 2 % *Curcuma longa*; T3: 25 % hempseed and 2 % *Zingiber officinale*.

chromatography (GC) vial. Complete fatty acids methyl esters were separated by adding 1 mL hexane and vortexed for 2 min followed by centrifugation ($800 \times g$ for 5 min). The fatty acid layer was transferred into GC vials and analysed with gas chromatography mass spectrometry (Shimadzu-QP 2010 Plus, Japan) with a fused silica capillary column with 50 m × 0.250 mm and 0.2 µm film thickness (Supelco, SP-2560; Supelco, Bellefonte PA, USA) using hydrogen as a carrier gas. One microlitre of the sample was injected into the GC with a split ratio of 1 : 5. The flame ionization detector was set to 280 °C. The time–temperature program used started with an initial temperature of 140 °C for 4 min, increased by 4 °C per minute to a final temperature of 240 °C and was held at this temperature for 20 min. The fatty acid methyl esters were identified using external standards, and the fatty acid contents were calculated from the peak area of the corresponding fatty acid in relation to the total area of all peaks.

2.3 Statistical analysis

Statistical data were analysed using standard methods of one-way analysis of variance (ANOVA) in a completely random-

Table 2. Mean ±SE saturated fatty acid (mg g^{-1} of yolk) in eggs from hens fed with and without natural herbs

Experimental groups	Control	T1	T2	T3
Myristic (C14 : 0)				
Day 0	$0.79^{a} \pm 0.03$	$0.55^{b} \pm 0.03$	$0.54^{b} \pm 0.02$	$0.52^{b} \pm 0.02$
Day 15	$0.61^{a} \pm 0.04$	$0.57^{b} \pm 0.02$	$0.52^{b} \pm 0.02$	$0.51^{b} \pm 0.01$
Day 30	$0.62^{a} \pm 0.02$	$0.64^{a} \pm 0.02$	$0.52^{b} \pm 0.01$	$0.55^{b} \pm 0.02$
Palmitic (C16 : 0)				
Day 0	$54.97^{a} \pm 0.55$	$34.82^{b} \pm 0.49$	$34.20^{b} \pm 0.65$	$33.82^{b} \pm 0.04$
Day 15	$40.95^{a} \pm 0.49$	$36.21^{b} \pm 0.66$	$34.60^{c} \pm 0.35$	$33.63^{c} \pm 0.28$
Day 30	$40.33^{a} \pm 0.33$	$36.22^{b} \pm 0.65$	$35.32^{c} \pm 0.33$	$34.65^{bc} \pm 0.34$
Stearic (C18 : 0)				
Day 0	$19.57^{a} \pm 0.80$	$15.78^{b} \pm 0.29$	$15.56^{b} \pm 0.29$	$15.77^{b} \pm 0.10$
Day 15	$20.06^{a} \pm 0.10$	$17.22^{b} \pm 0.09$	$16.17^{c} \pm 0.09$	$15.75^{c} \pm 0.08$
Day 30	$20.54^{a} \pm 0.21$	$15.93^{b} \pm 0.26$	$15.49^{b} \pm 0.26$	$15.67^{b} \pm 0.26$
Total SFA				
Day 0	$62.33^{a} \pm 0.78$	$51.15^{b} \pm 0.61$	$50.28^{b} \pm 0.94$	$50.13^{b} \pm 0.17$
Day 15	$58.82^{a} \pm 0.36$	$54.00^{b} \pm 0.91$	$51.28^{c} \pm 0.26$	$49.88^{c} \pm 0.34$
Day 30	$56.80^{a} \pm 0.56$	$53.09^{b} \pm 0.71$	$50.66^{bc} \pm 0.49$	$51.54^{c} \pm 0.28$

Mean values bearing different superscripts differ significantly ($P < 0.05$); SFA: saturated fatty acids; T1: hempseed; T2: 25 % hempseed and 2 % *Curcuma longa*; T3: 25 % hempseed and 2 % *Zingiber officinale*.

Table 3. Mean ±SE monounsaturated fatty acid (mg g^{-1} of yolk) in eggs from hens fed with and without natural herbs.

Experimental groups	Control	T1	T2	T3
Palmitoleic acid				
Day 0	$2.95^{a} \pm 0.04$	$2.40^{b} \pm 0.13$	$2.32^{b} \pm 0.15$	$2.40^{b} \pm 0.21$
Day 15	$2.95^{a} \pm 0.05$	$3.03^{b} \pm 0.04$	$2.36^{c} \pm 0.13$	$2.33^{c} \pm 0.16$
Day 30	$2.97^{a} \pm 0.03$	$3.89^{b} \pm 0.14$	$2.48^{c} \pm 0.08$	$2.49^{c} \pm 0.08$
Oleic acid				
Day 0	$55.70^{a} \pm 0.52$	$51.00^{b} \pm 0.58$	$51.02^{b} \pm 0.57$	$51.03^{b} \pm 0.61$
Day 15	$56.39^{a} \pm 0.14$	$52.50^{b} \pm 0.46$	$51.08^{b} \pm 0.58$	$51.04^{b} \pm 0.61$
Day 30	$57.05^{a} \pm 0.18$	$53.05^{b} \pm 0.67$	$51.20^{c} \pm 0.60$	$51.43^{c} \pm 0.31$
MUFA (total)				
Day 0	$57.65^{a} \pm 0.52$	$53.40^{b} \pm 0.53$	$53.33^{b} \pm 0.70$	$53.43^{b} \pm 0.79$
Day 15	$61.33^{a} \pm 0.11$	$55.53^{b} \pm 0.41$	$53.44^{c} \pm 0.68$	$53.37^{c} \pm 0.71$
Day 30	$62.03^{a} \pm 0.16$	$56.95^{b} \pm 0.80$	$53.92^{c} \pm 0.64$	$53.68^{c} \pm 0.33$

Mean values bearing different superscripts differ significantly ($P < 0.05$); T1: hempseed; T2: 25 % hempseed and 2 % *Curcuma longa*; T3: 25 % hempseed and 2 % *Zingiber officinale*; MUFA: monounsaturated fatty acid.

ized design. Means were separated by a least significant difference test. The statistics software STATISTIX (8.1) was used for data analysis. P values less than 0.05 were considered statistically significant.

3 Results

The concentration of saturated fatty acids is given in Table 2. The results indicated that individual and total fatty acids were significantly lower ($P < 0.05$) in the experimental groups by day 0, 15, and 30 compared to the control.

The entire monounsaturated fatty acid profile was significantly ($P < 0.05$) influenced by supplementation with or

Table 4. Mean ±SE polyunsaturated fatty acids (mg g^{-1} of yolk) in eggs from hens fed with and without natural herbs.

Experimental groups	Control	T1	T2	T3
Linoleic acid (*n*-6)				
Day 0	$45.44^{b} \pm 0.34$	$56.37^{a} \pm 0.31$	$56.42^{a} \pm 0.30$	$56.36^{a} \pm 0.33$
Day 15	$50.63^{c} \pm 0.29$	$54.50^{b} \pm 0.28$	$56.45^{a} \pm 0.29$	$56.39^{a} \pm 0.33$
Day 30	$51.31^{c} \pm 0.29$	$53.16^{b} \pm 0.44$	$56.47^{a} \pm 0.31$	$56.41^{a} \pm 0.33$
Eicosadienoic acid (*n*-6)				
Day 0	$0.34^{a} \pm 0.00$	$0.34^{a} \pm 0.00$	$0.34^{a} \pm 0.00$	$0.33^{a} \pm 0.00$
Day 15	$0.05^{c} \pm 0.00$	$0.30^{b} \pm 0.00$	$0.32^{a} \pm 0.00$	$0.31^{ab} \pm 0.00$
Day 30	$0.05^{c} \pm 0.00$	$0.27^{b} \pm 0.00$	$0.30^{a} \pm 0.00$	$0.29^{a} \pm 0.00$
Arachidonic acid (*n*-6)				
Day 0	3.45 ± 0.03	3.49 ± 0.03	3.56 ± 0.03	3.59 ± 0.03
Day 15	3.44 ± 0.03	3.51 ± 0.06	3.55 ± 0.03	3.58 ± 0.03
Day 30	3.47 ± 0.03	3.51 ± 0.05	3.55 ± 0.03	3.58 ± 0.03
PUFA (total)				
Day 0	$36.96^{b} \pm 0.46$	$73.84^{a} \pm 0.53$	$73.98^{a} \pm 0.91$	$73.95^{a} \pm 1.00$
Day 15	$64.06^{c} \pm 0.40$	$70.64^{b} \pm 0.52$	$73.74^{a} \pm 0.76$	$73.73^{a} \pm 0.88$
Day 30	$65.72^{c} \pm 0.21$	$68.31^{b} \pm 0.75$	$73.21^{a} \pm 0.62$	$73.18^{a} \pm 0.76$

Mean values bearing different superscripts differ significantly ($P < 0.05$); SFA: saturated fatty acids; T1: hempseed; T2: 25 % hempseed and 2 % *Curcuma longa*; T3: 25 % hempseed and 2 % *Zingiber officinale*.

Table 5. Mean ±SE monounsaturated fatty acid (mg g^{-1}) in eggs from hens fed with and without natural herbs.

Experimental groups	Control	T1	T2	T3
Linolenic acid (*n*-3)				
Day 0	$7.39^{a} \pm 0.09$	$8.73^{a} \pm 0.37$	$8.75^{a} \pm 0.37$	$8.75^{a} \pm 0.37$
Day 15	$5.41^{c} \pm 0.10$	$7.53^{b} \pm 0.20$	$8.58^{a} \pm 0.28$	$8.57^{a} \pm 0.27$
Day 30	$5.45^{c} \pm 0.09$	$6.62^{b} \pm 0.12$	$8.08^{a} \pm 0.05$	$8.25^{a} \pm 0.24$
Eicosatrienoic acid (*n*-3)				
Day 0	$0.18^{a} \pm 0.00$	$0.20^{a} \pm 0.00$	$0.20^{a} \pm 0.00$	$0.21^{a} \pm 0.00$
Day 15	$0.08^{b} \pm 0.00$	$0.18^{a} \pm 0.00$	$0.18^{a} \pm 0.00$	$0.19^{a} \pm 0.00$
Day 30	$0.05^{b} \pm 0.00$	$0.16^{a} \pm 0.00$	$0.17^{a} \pm 0.00$	$0.17^{a} \pm 0.00$
EPA (*n*-3)				
Day 0	$0.41^{a} \pm 0.05$	$0.43^{a} \pm 0.05$	$0.44^{a} \pm 0.05$	$0.44^{a} \pm 0.05$
Day 15	$0.07^{b} \pm 0.03$	$0.44^{a} \pm 0.05$	$0.44^{a} \pm 0.05$	$0.44^{a} \pm 0.05$
Day 30	$0.05^{b} \pm 0.04$	$0.45^{a} \pm 0.05$	$3.47^{a} \pm 0.05$	$3.47^{a} \pm 0.06$
DHA (*n*-3)				
Day 0	$4.38^{a} \pm 0.03$	$4.28^{a} \pm 0.36$	$4.28^{a} \pm 0.35$	$4.28^{a} \pm 0.36$
Day 15	$2.35^{b} \pm 0.03$	$4.18^{a} \pm 0.31$	$4.22^{a} \pm 0.34$	$4.26^{a} \pm 0.37$
Day 30	$2.34^{b} \pm 0.04$	$4.14^{a} \pm 0.05$	$4.19^{a} \pm 0.05$	$4.01^{a} \pm 0.06$

Mean values bearing different superscripts differ significantly (P<0.05); T1: hempseed; T2: 25 % hempseed and 2 % *Curcuma longa*; T3: 25 % hempseed and 2 % *Zingiber officinale*; EPA: eicosapentaenoic acid; DHA: docosahexaenoic acid.

Table 6. Mean ±SE total ω-3 (mg g^{-1}), total ω-6 (mg g^{-1}) and ω-6 : ω-3 in eggs from hens fed with and without natural antioxidants.

Experimental groups	Control	T1	T2	T3
Total ω-3				
Day 0	$12.96^{a} \pm 0.16$	$13.64^{a} \pm 0.63$	$13.66^{a} \pm 0.63$	$13.67^{a} \pm 0.64$
Day 15	$11.91^{b} \pm 0.15$	$12.33^{a} \pm 0.47$	$13.42^{a} \pm 0.49$	$13.46^{a} \pm 0.52$
Day 30	$10.90^{c} \pm 0.14$	$11.37^{b} \pm 0.46$	$12.89^{a} \pm 0.35$	$12.90^{a} \pm 0.45$
Total ω-6				
Day 0	$64.00^{a} \pm 0.32$	$60.20^{a} \pm 0.33$	$60.32^{a} \pm 0.29$	$60.28^{a} \pm 0.36$
Day 15	$54.16^{c} \pm 0.28$	$58.31^{b} \pm 0.26$	$60.32^{a} \pm 0.27$	$60.28^{a} \pm 0.36$
Day 30	$51.83^{c} \pm 0.27$	$56.94^{b} \pm 0.41$	$60.31^{a} \pm 0.29$	$60.28^{a} \pm 0.35$
ω-6 : ω-3				
Day 0	$4.55^{a} \pm 0.57$	$4.43^{a} \pm 0.21$	$4.43^{a} \pm 0.17$	$4.42^{a} \pm 0.17$
Day 15	$6.81^{a} \pm 0.59$	$4.47^{b} \pm 0.19$	$4.51^{b} \pm 0.14$	$4.49^{b} \pm 0.14$
Day 30	$7.09^{a} \pm 0.68$	$5.02^{b} \pm 0.19$	$4.69^{b} \pm 0.11$	$4.69^{b} \pm 0.14$

Means values bearing different superscripts differ significantly ($P < 0.05$); T1: hempseed; T2: 25 % hempseed and 2 % *Curcuma longa*; T3: 25 % hempseed and 2 % *Zingiber officinale*.

without treatments (Table 3). By day 0, 15, and 30, total monounsaturated fatty acids were significantly low ($P < 0.05$) in T3 compared to the control. Similarly, palmitoleic acid and oleic acid decreased significantly ($P < 0.05$) in T3 compared to the control group. Total polyunsaturated fats were significantly ($P < 0.05$) affected by the enrichment of diets with natural herbs (Table 4). In fresh eggs, polyunsaturated fatty acids (PUFAs) increased significantly in T2 and T3 and were lower in the control and T1 by day 15 and 30. The same trend was also found in linoleic, eicosadienoic, and arachidonic acid.

Linolenic acid, eicosatrienoic acid, eicosapentaenoic acid, and docosahexaenoic acid are shown in Table 5. The result indicated that linolenic acid increased significantly ($P < 0.05$) in T2 and T3 by day 15 and 30. However, eicosatrienoic acid, eicosapentaenoic acid, and docosahexaenoic acid increased significantly ($P < 0.05$) in all treated groups compared to the control by day 15 and 30.

Total ω-3 and ω-6 and their ratio has been presented in Table 6. By day 15 and 30, total ω-3 increased significantly in treated groups. In contrast to ω-3, ω-6 increased significantly in T2 and T3 by day 15 and 30, while their ratio decreased significantly in the treated groups.

4 Discussion

Classic eggs typically contain a low level of the n-3 PUFA and therefore contribute little to meeting the daily recommended intake (Neijat et al., 2015). The fatty acid profile of egg yolk could be altered by adding natural sources of n-3 PUFA into the diet of the hen (Baucells et al., 2000). Contrary to the potential of industrial hempseed, its use in the poultry diet is not recommended due to a lack of scientific evidence to support nutritional efficacy (Gakhar et al., 2012). Previous reports have shown that the addition of hempseed can potentially generate n-3-enriched eggs (Silversides and Lefrançois, 2005; Gakhar et al., 2012; Neijat et al., 2015). In the present study, the results are encouraging since the profile of fatty acids improved significantly. Further, in the current study, entire saturated fatty acid and individual saturated fatty acid (like myristic acid, palmitic acid, and stearic acid) values were lower, especially in T2 and T3. Saturated fatty acids increase the risk of heart disease, so lower consumption is recommended. A reduced concentration of saturated fats during 15 and 30 days in herb-fed groups as compared those not fed herbs may be due to the antioxidant properties of turmeric and ginger (Sreejayan, 1994; Ruby et al., 1995; Dieumou et al., 2009). Cherian et al. (2007) reported lower monounsaturated fats (during storage) in the yolk of hens receiving different levels of conjugated linoleic acid in their feed, which supports our results. In contrast to our results, Hayat et al. (2010) reported a higher concentration of monounsaturated fatty acids during storage in ω-3-enriched diets with flaxseed and antioxidants.

Arachidonic acid (n-6) was not significantly ($p < 0.05$) affected in all the experimental groups during the storage of eggs at room temperature. In contrast with our results, Hayat et al. (2010) reported decreased egg yolk arachidonic acid. Eicosadienoic acid (n-6) was significantly influenced in hempseed-fed groups enriched with natural herbs during storage. Highest eicosadienoic fats in fresh and stored eggs were recorded in groups T2 and T3 and lowest in the control group. Hempseed has 0.09 % eicosadienoic acid (Deferne and Pate, 1996; Callaway and Laakkonen, 1996); a part of this may be channelled into the egg after metabolizing in the liver.

Linolenic acid (n-3) concentration was significantly affected by hempseed-fed groups that either did or did not receive natural herbs. An increased level of linolenic acid was found in T2 and T3, while the lowest value was recorded in a positive control and negative control group in stored eggs. Our results correspond to those of Cherian et al. (2007), who found decreased levels of linoleic acid in the egg yolk of laying hens during 60 days of storage in ω-3-enriched diets.

Total ω-3 polyunsaturated fatty acids were altered significantly among different groups. In analysed eggs on day 0, 15, and 30, total ω-3 fatty acids were reported to be higher for groups receiving hempseed, hempseed with 2 % turmeric, and hempseed with 2 % ginger, while the lowest value was found in the group without hempseed and natural herbs. During storage for 30 days, the highest total n-3 was reported in T2 and T3. An increase in total ω-3 fats in turmeric- and ginger-fed groups may be due to the antioxidant profile of ginger and turmeric (Ruby et al., 1995; Dieumou et al., 2009), which can regulate the elongase and desaturase pathway in a favourable way during egg yolk lipid metabolism. In agreement with our results, Cherian et al. (2007) reported a decreased level of total ω-3 in the egg yolk of laying hens during 60 days of storage in ω-3-enriched diets without natural antioxidants.

Total ω-6 fatty acids were significantly influenced by the inclusion of various antioxidants in ω-3-enriched diets. Antioxidant properties of herbs can stimulate the metabolic pathway of essential fatty acids like ω-3 and ω-6 (Hayat et al., 2010). The best ω-6 : ω-3 ratio was recorded in the groups consuming hempseed, hempseed with 2 % turmeric, and hempseed with 2 % ginger, while the lowest ratio was found in the control group. Antioxidant activities of natural antioxidants like ginger and turmeric can alter the metabolic pathway of essential fatty acids like ω-3 and ω-6 (Hayat et al., 2010) and ultimately lower the ω-6 : ω-3 ratio, which is ideal for human wellbeing.

In summary, compared to the control, the addition of hempseed at the level of 25 % alone or in combination with 2 % ginger and turmeric in the diet of Hy-Line White Leghorn improved n-3 polyunsaturated fatty acids and the ratio of ω-3 and ω-6 and decreased saturated fatty acids in the yolk of eggs stored at room temperature for 30 days.

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