

## Comparative Analysis of Fatty Acid Composition of Yolk Lipids in Indigenous and Conventional Chicken Eggs

Research Article

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### ABSTRACT

Eggs of the indigenous laying hens are preferred by consumers to those obtained from commercial breeds. The present study aimed to compare the fatty acid (FA) composition of yolk lipids of indigenous eggs (Indigenous;  $n = 20$ ) and conventional eggs (Conventional;  $n = 20$ ). Indigenous and conventional eggs were collected from Lori layer hens reared under free-range condition and an commercial farm respectively. The FA composition of yolk lipids in indigenous and conventional eggs was determined by gas chromatography. Lori eggs (48.7 g) were significantly lighter than conventional eggs (59.6 g). Total fatty acid content (g/100 g fresh yolk) was similar in Lori and conventional egg yolk with 27.7 and 28.6 respectively. Lori eggs had significantly higher docosahexaenoic (DHA, C22:6n3),  $\alpha$ -linoleic (ALA, C18:3n3) and oleic acid (C18:1n9cis) levels and lower linoleic (C18:2n6cis) levels than conventional eggs. Lori eggs had significantly higher content of  $n$ -3 and lower content of  $n$ -6 FA than the conventional eggs. In conclusion, the  $n$ -6/ $n$ -3 ratio in Lori eggs (8.1) was significantly lower than that in conventional eggs (17.4) proposing that indigenous eggs may be healthier for the consumers than the conventional eggs.

**KEY WORDS** fatty acids, indigenous hen, Lori egg, omega-3.

### INTRODUCTION

Rural Indigenous chicken production in Iran goes back a long way (Shariatmadari, 2000; Vali, 2008). Local poultry production plays an important role in livelihood of the farmers not only for their products (meat, eggs) but also for improving farmers' income (Mwalusanya *et al.* 2002). Indigenous laying hens are usually kept in free-range condition and they graze mainly green plants and scavenge for earthworms (Mwalusanya *et al.* 2002). It has been previously reported that FA composition of eggs was affected by laying hens diet (Beynen, 2004; Milinsk *et al.* 2003), farming conditions (Rizzi *et al.* 2007) and housing system (Hidalgo *et al.* 2008; Lopez-Bote *et al.* 1998). Furthermore, studies have shown that FA composition of the egg was

also influenced by the laying hen breed (Millet *et al.* 2006; Scheideler *et al.* 1998). Eggs of indigenous laying hens differ from that of commercial hens in term of breed, housing condition, and diets. Consumers mainly prefer eggs of the indigenous laying hens rather than those obtained from commercial breeds. While fatty acid composition of conventional eggs is well studied (Cherian *et al.* 2002; Samman *et al.* 2009), data regarding the FA composition of eggs produced by local indigenous breeds are limited. The Lori laying hen is an indigenous breed reared by villagers in the west of Iran, mainly in Lorestan province. The Lori layer hen is a farmyard breed and rears under free-range conditions. The Lori hen produces about 80 to 100 brown shell eggs. In spite of favorability of Lori eggs for the consumers, actual FA composition of Lori eggs has not yet

been determined. Thus, the objectives of this study were to determine the FA composition and to compare *n-6/n-3* ratio of yolk lipids of indigenous Lori eggs to that of the conventional eggs.

## MATERIALS AND METHODS

### Sample collection and preparation

Indigenous eggs were collected from Lori layer hens kept under free-range condition in a village near Khorramabad city, Iran. It is a semi-dried area with average annual rainfall of about 472 mm, an altitude of 1125 m and average temperature of 17.3 °C. The Lori hens grazed fresh green grass and legumes *ad libitum*. The predominant plant species of the ranges were ryegrass (*Lolium perenne*) and clover (*Trifolium* spp.). Lori hens habitually scavenge earthworms. Conventional eggs were collected from an industrial laying hen farm (Leghorn breed). In total, 40 eggs were collected (20 Lori eggs and 20 commercial Lohmann selected Leghorn eggs). All eggs were weighed and the yolks were separated from the whites and frozen at -20 °C pending analysis.

### Lipid extraction and FA methylation

Fatty acid methyl esters (FAME) were determined using the procedures described by [Sukhija and Palmquist \(1988\)](#) with some modification. Briefly, about 0.1 g yolk was weighed by digital scale (0.0001, KERN, Germany) and then was dried by means of freeze dryer for 48 h. Freeze-dried samples were weighed again after drying and were placed into culture tube (16×125 mL, Scott glass tube). About one mL heptane including internal standard (C13:0) was added and mixed. Then 0.2 mL of sodium methylate (25%) was added and the tube was put in a 50 °C water bath for 10 min. After that, sample was cooled for about 5 min. Then 3 mL of freshly made methanolic HCl 10% (prepared by adding 20 mL of acetyl chloride to 100 mL of anhydrous methanol) was added and vortexed. The tube was put in a 90 °C steam bath for 30 min and then the tube sample was cooled. Finally, one mL of heptane and three mL of potassium carbonate 10% was added and mixed for one min. The sample was centrifuged (Centrifuge 5415 R; Rotofix 32A, Germany) for 5 min at 1500 g, and Heptane phase (upper phase) and was transferred to the GC vial (1.5 mL) using a pastor pipette.

### Determination of FA composition

FAME were analyzed by gas chromatography with flame ionization detection (GC-FID; HP 6890 chromatograph, Hewlett-Packard, Avondale, PA, USA) using a Chrompack CP-Sil 88 TM fused silica capillary column (100 m×0.25 mm internal diameter, 0.2 mm film thickness; Varian Inc., Walnut Creek, CA, USA). Briefly, the oven temperature

was initially 150 °C (held for 5 min), then increased for 5 °C/min to 180 °C (held for 30 min), then increased by 1 °C/min to 190 °C (held for 5 min) and finally increased by 1 °C/min to 200 °C (held for 35 min). Hydrogen was used as the carrier gas at a flow rate of 1.0 mL/min. The injector and detector temperatures were maintained at 280 and 300 °C respectively. Identification of common FA was accomplished by comparison of sample peak retention times with those of FAME standard mixtures (Supelco™ 37 component FAME Mix, Supelco-47885-U, Sigma-Aldrich Chemie GmbH, Germany). Quantification of total FAME was calculated using tridecanoic acid (C13:0) as internal standard (Fluka-91988, Sigma-Aldrich Chemie GmbH, Germany). The FA content of yolk egg was calculated as concentration (mg/g) = peak area of a given FA × concentration of internal standard (mg/mL) / peak area of internal standard / sample weight (g).

### Statistical analysis

Data were analyzed by SAS/STAT software ([SAS, 2001](#)) using t-test procedure. Normality of residuals was tested using a Shapiro-Wilk test. Statistical differences with ( $P < 0.05$ ) were considered significant.

## RESULTS AND DISCUSSION

The weight of indigenous Lori eggs were lower compared to the conventional white Leghorn eggs (48.7±3.6 vs. 59.6±3.2,  $P < 0.01$ ). The weight of the Lori eggs was in line with the reported range of eggs weigh for the other Iranian indigenous laying hens ([Vali, 2008](#)). In agreement with the finding of the present study, Araucana (a fancy breed) hens produced lighter egg compared to the commercial breeds ([Millet et al. 2006](#)). Basically, eggs weight is determined by laying hen breed, feed intake, and housing conditions ([Hughes et al. 1985](#); [Millet et al. 2006](#)). Thus, the lower weight of indigenous eggs compared to the conventional eggs could be partly explained by feed intake and the type of breed. The FA compositions of indigenous Lori and conventional egg yolks both as percentage (wt.%) and as content (g) in 100 g fresh yolk are shown in Table 1 and Table 2 respectively. In Lori eggs similar to the commercial eggs, palmitic acid (C16:0) and stearic acid (C18:0) were the predominant saturated FA, which was in agreement with the previously reported values ([Hidalgo et al. 2008](#); [Samman et al. 2009](#); [Simčič et al. 2011](#)). Lori egg yolk had about 20% higher stearic acid (C18:0) than the conventional egg (9.98 vs. 8.43 wt.%) albeit total saturated FA (wt.%) did not differ between two type of eggs. The higher stearic acid in indigenous egg might be due the fact that Lori hens consumed animal fats with high content of stearic acid found in worms ([Hansen and Czochanska, 1975](#)) and insects ([Raksakantong et al. 2010](#)).

**Table 1** Fatty acid composition of yolk (wt. %) of indigenous Lori and white Leghorn hens reared under indigenous and conventional methods respectively

Items	Conventional eggs (n=20)		Indigenous eggs (n=20)		t-test
	Mean	±SD	Mean	±SD	
Myristic acid (C14:0)	0.37	0.05	0.44	0.21	NS
Palmitic acid (C16:0)	26.0	1.53	25.8	1.34	NS
Margaric acid (C17:0)	0.18	0.07	0.30	0.07	NS
Stearic acid (C18:0)	8.43	0.65	9.98	1.10	**
Total saturated fatty acids	35.7	1.25	36.6	1.70	NS
Palmitoleic (C16:1 n-9)	3.59	0.76	3.35	0.83	NS
Oleic acid (C18:1 n-9)	39.3	2.05	41.5	2.20	*
Linoleic (C18:2 n-6)	12.7	1.40	9.28	1.51	**
α-Linolenic acid (C18:3 n-3)	0.33	0.04	0.53	0.25	*
Arachidonic acid (C20:4 n-6)	2.04	0.08	1.75	0.42	NS
Docosahexaenoic (C22:6 n-3)	0.52	0.11	0.90	0.23	***
Trans fatty acids	4.90	0.60	4.70	0.9	NS
Monounsaturated fatty acids	48.1	2.36	50.0	2.55	NS
Total unsaturated fatty acids	64.2	1.26	63.4	1.70	NS
Unsaturated / saturated ratio	1.80	0.10	1.70	0.13	NS
Total n-6 family	15.2	1.72	11.8	1.70	***
Total n-3 family	0.89	0.14	1.62	0.56	***
n-6 / n-3 ratio	17.4	2.48	8.1	3.10	***

n: number of samples and SD: standard division.

\* (P<0.05); \*\* (P<0.01) and \*\*\* (P<0.001).

NS: non significant.

**Table 2** Fatty acid content of yolk egg (g/100 g yolk) of indigenous Lori and white Leghorn hens reared under indigenous and conventional methods respectively

Items	Conventional eggs (n=20)		Indigenous eggs (n=20)		t-test
	Mean	±SD	Mean	±SD	
Myristic acid (C14:0)	0.10	0.03	0.12	0.05	NS
Palmitic acid (C16:0)	7.51	1.60	7.16	1.03	NS
Stearic acid (C18:0)	2.39	0.60	2.78	0.52	NS
Total saturated fatty acids	10.1	2.20	10.2	1.50	NS
Palmitoleic (C16:1 n-9)	1.00	0.27	0.93	0.25	NS
Oleic acid (C18:1 n-9)	11.1	2.60	11.5	1.48	NS
Linoleic (C18:2 n-6)	3.60	0.90	2.61	0.66	**
α-linolenic acid (C18:3 n-3)	0.09	0.02	0.15	0.07	*
Arachidonic acid (C20:4 n-6)	0.58	0.17	0.49	0.13	NS
Docosahexaenoic (C22:6 n-3)	0.15	0.04	0.25	0.08	***
Trans fatty acids	1.4	0.37	1.3	0.25	NS
Total unsaturated fatty acids	18.1	4.04	17.6	2.36	NS
Monounsaturated fatty acids	13.6	3.10	13.8	1.60	NS
Total n-6 family	4.30	1.10	3.30	0.78	*
Total n-3 family	0.25	0.06	0.45	0.18	**
Total fatty acid	28.2	6.0	27.7	3.7	NS

n: number of samples and SD: standard division.

\* (P<0.05); \*\* (P<0.01) and \*\*\* (P<0.001).

NS: non significant.

The higher stearic acid in Lori eggs compared to the conventional eggs could have health beneficial effects for the consumers, because stearic acid has a neutral effect on diet-related disease risk (Baum, *et al.* 2012).

Arachidonic acid (C20:4 n-6) and docosahexanoic acid (C22:6 n-3) were the most abundant long chain unsaturated FA in both indigenous and conventional eggs. Lori egg yolks had significantly higher α-linolenic acid (ALA, C18:3 n-3) and higher docosahexanoic acid (DHA, C22:6 n-3) but lower linoleic acid (LA, C18:2 n-6) than the conventional egg yolks.

When values are presented as g per 100 g fresh yolk, Lori yolk eggs had twice much as high docosahexanoic acid (C22:6 n-3, 250 vs. 150 mg/100 g yolk) and relatively higher α-linolenic acid (C18:3 n-3) compared to the conventional eggs. On the other hand, conventional eggs had higher linoleic acid (C18:2 n-6). Consequently, Lori egg yolks had higher n-3 FA (400 vs. 200 mg/100g yolk) levels but lower n-6 FA (3.3 vs. 4.3 g/100 g yolk) levels than the conventional egg yolks. In agreement with the present results, eggs from Araucana compared to commercial Lohmann selected Leghorn had about 120 mg/100 g yolk

higher docosahexanoic acid (Millet *et al.* 2006). Higher values of n-3 fatty acids found in Lori yolk compared to the conventional eggs might be due to the fact that Lori laying hens grazed on fresh green grass and legumes. Fresh green plants contain higher proportion of polyunsaturated FA especially  $\alpha$ -linoleic acid than the commercial mixed diet (Lopez-Bote *et al.* 1998). The eggs of white Leghorn laying hens fed with grass and a commercial mixed diet under free-range conditions had more n-3 FA as well as  $\alpha$ -Linolenic acid and docosahexanoic acid than the eggs of the hens fed only with a commercial diet (Lopez-Bote *et al.* 1998).

Interestingly, the n-6/n-3 ratio in the Lori eggs (8.1) was lower than that in the conventional eggs (17.4). The lower ratio of n-6/n-3 in Lori egg compared to the value in commercial egg is considered healthier from the nutritional point of view.

In the present study, the n-6/n-3 ratio found in the conventional egg was in good agreement with previously reported value for market egg (Samman *et al.* 2009). The n-6/n-3 ratio in human nutrition is important for human health (Simopoulos, 2008).

A lower n-6/n-3 ratio of FA is desirable due to the links between unbalanced n-6/n-3 ratio and prevalence of many of the human chronic diseases (Simopoulos, 2008). The evidence have shown that there are relations between unbalanced n-6/n-3 ratio and cardiovascular disease, cancer, insulin resistance, depression, early aging, diabetes, and obesity (Adkins and Kelley, 2010; Bourre, 2004; Simopoulos, 2008).

In recent decades, n-6 FA consumption had increased by 250%, while n-3 FA had decreased by 30% in the human diets (Kouba and Mouro, 2011). Findings of the present study revealed that indigenous Lori layer hens produce eggs with healthier n-6/n-3 ratio compared to market eggs (commercial leghorn eggs). However, it should be mentioned that again the good nutritional characteristics of indigenous eggs might be due to the hens' diet rather than the hens' breed. In conventional production system, hens are fed with formulated mixed feeds, whereas indigenous breeds had free access to the green forages. Basically, green forages have high content of LA (7 to 37 g), ALA (2 to 10 g) and palmitic acid (3 to 8 g) (Clapham *et al.* 2005) and most likely, secondary metabolites in legumes may affect FA metabolism. Karsten *et al.* (2010) reported that eggs of pastured hens had twice as much n-3 fatty acids, and 2.5 fold more total n-3 fatty acids compared to eggs of caged hens (Karsten *et al.* 2010). Further research is needed to determine whether the healthy n-6/n-3 ratio found in indigenous eggs was due to the laying hens' diets or to the breed per se.

## CONCLUSION

This study showed that eggs produced by indigenous Lori layers reared under free-range condition had higher stearic acid and higher n-3 fatty acids compared to the market eggs. The n-6/n-3 ratio in Lori eggs (8.1) was significantly lower than that in conventional eggs (17.4) proposing that indigenous eggs may be healthier for the consumers than the conventional eggs.

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