Egg Fortification with n-3 Polyunsaturated Fatty Acids (PUFA): Nutritional Benefits versus High n-6 PUFA Western Diets, and Consumer Acceptance

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Abstract

Background: As high dietary n-6 polyunsaturated fatty acids and n-6:n-3 PUFA ratio may contribute to many western ailments, increasing n-3 PUFA in foods could be beneficial. The nutritional significance of n-3 PUFA-fortified egg vs. enzymatically competitive high n-6 PUFA diets is debatable.

Objectives: To evaluate the dietary contribution of 'field fortification' of eggs by adding n-3 PUFA to high n-6 PUFA hen feed and to assess whether it meets consumer preferences.

Methods: Laying hens (n=3500) were fed n-3 PUFA-fortified (5% extruded linseed) feed or standard (control) feed for 5 weeks. Nutritional significance was evaluated for western (American, Israeli) populations.

Results: Compared to regular (control) eggs, fortified eggs yielded a 3.8-fold increase in total n-3 PUFA, 6.4-fold alpha-linolenic acid (18:3), and 2.4-fold docohexaenoic acid 22:6). N-6:n-3 PUFA ratio decreased 3.6-fold, and n-6:n-3 long chain PUFA ratio (AA:DHA) 3.0-fold ($P < 0.0003$). Sensory evaluations were not significantly different. Egg cost increased by 1.5–3.0%. Fortified egg n-3 PUFA content averaged 14.3% of the current intake of Americans and 15.9% of Israelis – 9.8 and 10.6% of upper Dietary Reference Intakes, respectively. Egg DHA content averaged 33.7 and 41.4% of upper DRI. Current cholesterol intakes average 281 and 263 mg/day (median 214 and 184 mg/day) including 0.7 and 0.5 egg/day; reported hypercholesterolemia rates are 17.7 and 16.5%, respectively.

Conclusions: Effective concentration and transformation of supplemental n-3 PUFA/LCPUFA from feed to egg substantially enhanced egg n-3 PUFA %DRI, particularly of DHA, critical for health but often deficient. Such land-based n-3 PUFA/LCPUFA fortification may be applicable to high n-6 PUFA diets, fitting within cholesterol limitations and market criteria. It may contribute to general health and specific requirements (i.e., pregnancy and lactation), with possibilities of wide accessibility and standardization.

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It has been suggested that high dietary n-6 polyunsaturated fatty acids (PUFA) and n-6:n-3 PUFA ratio play a role in the high rates of chronic disease in western countries such as the United States, northern Europe, and Israel, in some cases despite a 'good' diet – namely, one that is balanced in total energy, fat, and saturated fat, and high in PUFA:SFA ratio, vegetables, and fruit [1-3]. Numerous epidemiological and clinical studies suggest a relationship between excessive n-6:n-3 PUFA ratio and risk of developing western pathologies [4]. In contrast, higher intake of n-3 PUFA may be beneficial in reducing risk in a number of conditions, including type 2 diabetes mellitus, neurological disorders and cardiovascular disease [2,4,5-8]. n-3 long-chain PUFA, specifically eicosapentaenoic acid (20:5 n-3) and docosahexaenoic acid (22:6 n-3), warrant special attention for these roles. They are involved in early-age organ development and function, especially the brain and nervous system, and compete with arachidonic acid (20:4 n-6) in metabolic pathways, reducing its resultant pro-inflammatory icosanoids [5,7]. However, n-3 PUFA are increasingly scarce in the western diet, and marine n-3 long chain PUFA even more so [8,9], with fish contamination presenting health risks [10].

As egg fatty acid profiles are highly dependent upon feed given to laying hens [8,11-13], they could become a significant source of n-3 PUFA and LCPUFA. High bioavailability of egg alpha-linolenic acid (18:3 n-3) and DHA has been shown by increased cellular DHA levels in consumers [14] as well as a reduced n-6:n-3 PUFA ratio [15] and a significant reduction in plasma triglycerides [8,16]. However, western eggs, i.e., American and Israeli, typically have a very high n-6 PUFA content relative to n-3 PUFA (n-6:n-3 PUFA ratio ≥ 17.4:1). Feed with high n-6 PUFA may competitively inhibit transformation of added n-3 PUFA, as both n-6 and n-3 PUFA are metabolized by the same enzyme [17].

This study addressed the feasibility of adding a vegetal (linseed) n-3 PUFA source to high n-6 PUFA hen feed to attain an egg that may contribute significantly to dietary fatty acid balance in high n-6 PUFA western diets, and that may be readily accepted by consumers.

PUFA = polyunsaturated fatty acids
DRI = Dietary Reference Intakes
LCPUFA = long chain PUFA (≥ C20)
DHA = docohexaenoic acid (22:6 n-3)

Materials and Methods

We randomly divided 3500 laying hens into two groups and fed them for 5 weeks with either a standard corn-based feed common to both the United States and Israel (control: Alonim, Israel), or the same feed fortified with 5% extruded linseed in accordance with the methods described by Weill et al. [15]. Standard feed yields 54% linoleic acid (18:2 n-6), and ELS mix yields 53 wt% alpha-linoleic acid. Egg fortification was performed by and under the auspices of Kibbutz Alonim Poultry Ltd. (Israel).

Feed and egg fatty acid and egg cholesterol analyses were performed at Lareal Laboratoire de Recherche Alimentaire (France) by fat extraction (Directive European 98/64/CE process B), preparation of esters by methylation (NF EN ISO 5509), and gas chromatographic separation and quantification (NF EN ISO 5508 June 1995/NF EN ISO June 2000).

n-3 PUFA contribution in egg was calculated by comparing control and fortified fatty acid content to dietary intake of American [18] and Israeli [19] populations, and to DRI (Institutes of Medicine, 2002) for adults aged ≥ 19 years. American and Israel DRI ranges for n-3 PUFA (including ALA, 0.6–1.2% kcal) and for LCPUFA (EPA + DHA 0.06–0.12% kcal) were calculated for men and women according to their average kcal intake. Egg cholesterol content was compared to DRI guidelines (≤ 300 mg/day) and to current consumption [18,19]. Blood cholesterol data for adults between the ages of 20 and 64 were referenced from American [20] and Israeli [21] epidemiological studies.

Sensory evaluation was performed by 12 blinded independent consumer volunteers (six women and six men aged ≥ 18) at Laboratoire Earning (Alfortville, France). They compared fortified to control eggs (five of each) according to the following criteria on a scale of 1–10: smell, taste, texture of yolk and white, viscosity, color, as well as general impression.

Statistical analyses of differences between eggs were performed using two-tailed paired *t*-tests, $\alpha = 0.05$.

Cost analysis was conducted by calculation of exchanging 5% ELS mix supplement with alternative sources of oils and other feed components (Alonim Ltd., Israel).

Table 1. FA profile of control and n-3 PUFA-fortified eggs (5% ELS), 5 week average

% FA	Total SFA	Total MUFA	Total PUFA	LA 18:2 n-6	AA 20:4 n-6	Total n-6 PUFA	ALA 18:3 n-3	DHA 22:6 n-3	Total n-3 PUFA	Total n-6:n-3 ratio	LA:ALA ratio	AA:DHA ratio
Control	34.7	44.3	21.1	16.5	1.9	19.6	0.4	0.7	1.2	16.3	41.3	2.7
	±0.4	±0.6	±0.6	±0.4	±0.1	±0.5	±0.0	±0.1	±0.1	±0.4	±1.1	±0.2
n-3 PUFA Fortified	32.6	42.3	25.2	18.3	1.5	20.5	2.6	1.7	4.5	4.5	7.2	0.9
	±0.4[a]	±0.6[b]	±0.7	±0.5[b]	±0.1[b]	±0.5[c]	±0.2[a]	±0.2[a]	±0.3[a]	±0.3[a]	±0.6[a]	±0.1

[a] $P < 0.0005$; [b] $P < 0.005$; [c] $P < 0.05$ vs. control
EPA was not detected

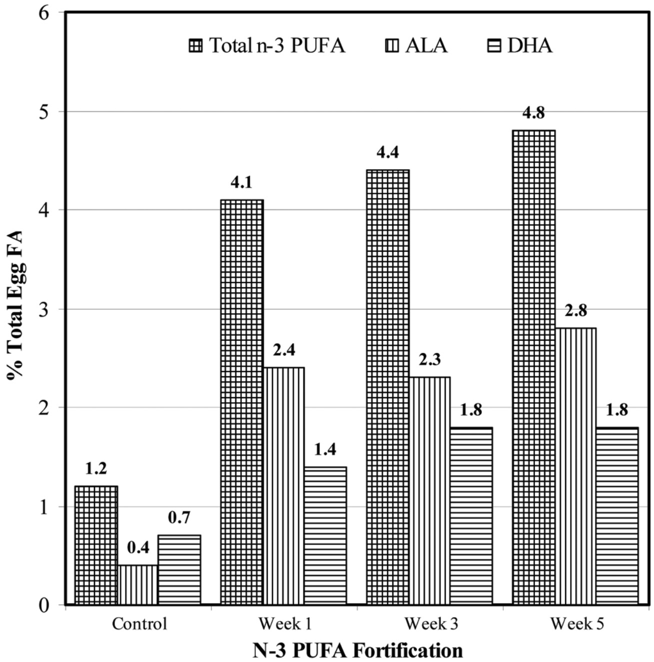

Figure 1. Patterns of change in n-3 PUFA %FA in control and fortified (5% ELS) eggs.

Results

In n-3 PUFA-fortified eggs, concentrations of total n-3 PUFA increased to 3.8-fold that of control eggs (258.2 vs. 67.3 mg/egg), ALA to 6.4-fold (156.7 vs. 24.5 mg), and DHA to 2.4-fold (101.6 vs. 42.8 mg). Correspondingly, total n-6:n-3 PUFA ratio was reduced 3.6-fold from the control, LA:ALA ratio 5.7-fold, and LCPUFA arachidonic acid:DHA ratio 3.0-fold [Table 1]. n-3 PUFA increased 3.4-fold already after the first week of fortification, followed by a continued and gradual increase to 3.7 and 4.0-fold after the third and fifth weeks respectively [Figure 1]. Saturated fatty acids, monounsaturated fatty acids, total PUFA, and n-6 PUFA contents were maintained; n-6 LCPUFA AA (20:4) was slightly reduced, to 0.79 of the control. The average cholesterol contents of control and fortified eggs were 218.8 and 212.5 mg respectively (not significant).

Current intake of total n-3 PUFA (as % of kcal/day) in American and Israeli men equals 0.8 and 1.0 kcal%, and is lower in women, 0.6 and 0.7 kcal% (vs. DRI range of 0.6–1.2 kcal%). The average LCPUFA (EPA + DHA) kcal% intake in American men and women averages 0.045 kcal% (vs. DRI range of 0.06–0.12 kcal%). Total n-3 PUFA in fortified eggs equaled approximately 7.1 and 10.9% of the upper DRI (1.2 kcal%) for American and Israeli men (vs. 2.5 and 2.7% DRI from control eggs), and for women 10.0 and 10.9% (vs. 2.6 and 2.8%). ALA equaled only 4.3 and 6.2% of the upper

ELS = extruded linseed
ALA = alpha-linolenic acid (18:3 n-3)
kcal = kilocalorie
LA = linolenic acid (18:2 n-6),

AA = arachidonic acid (20:4 n-6)

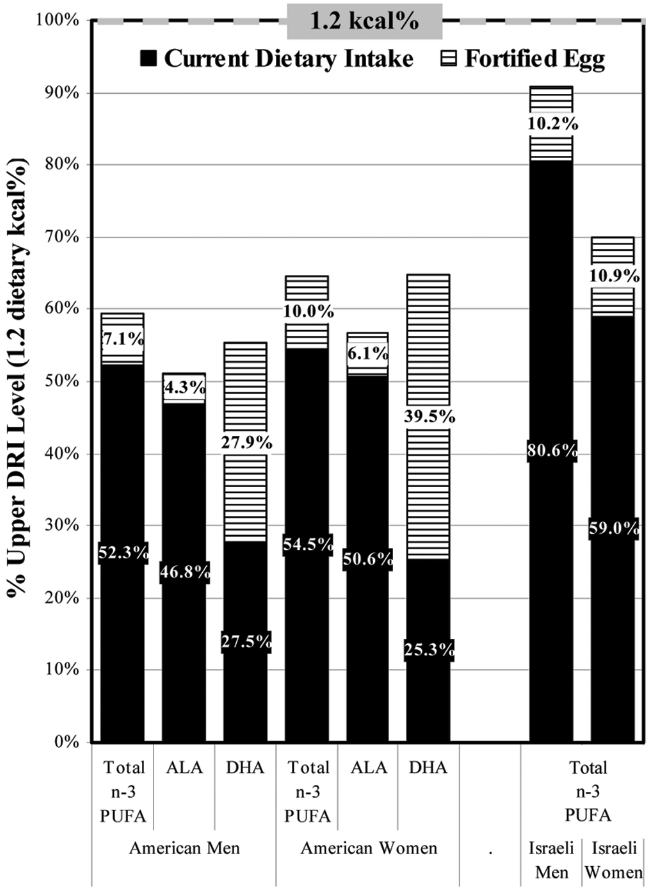

Figure 2. n-3 PUFA in current diets and in fortified eggs as %upper DRI (kcal%) levels calculated for American (total, ALA, DHA) and Israeli (total) men and women. DRI ranges: total n-3 PUFA and/or ALA = 0.6–1.2 kcal%, DHA = 0.06–0.12 kcal% of daily kcal intake for Americans (CDC, 2006) and Israelis (ICDC, 2003). *No information for current intake of individual n-3 PUFA among Israels is available.

DRI for men (vs. 0.7 and 1.0%), and for women 6.1 and 6.6% (vs. 1.0 and 1.1%). DHA was much higher, equaling approximately 27.9 and 39.9% of the upper DRI (0.12 kcal%) for American and Israeli men (vs. 11.8 and 16.8%), and for women 39.5 and 42.8% (vs. 16.7 and 17.8%) [Figure 2]. Substituting fortified for regular egg could reduce dietary n-6:n-3 PUFA ratio to 0.92 and 0.91 of current American and Israeli ratios, respectively.

Cholesterol intake in American adults (age 20–64) currently averages 281 mg/day (median 214 mg/day) with the intake of 0.7 egg/day [22]; women consume less: 237 and 177 mg/day [18]. Israeli adults consume 263 mg/day (median 185 mg/day) cholesterol with 0.5 egg/day; women consume less: 209 and 144 mg/day, respectively [19]. Hypercholesterolemia rates among Americans average 17.7% overall, 17.1% for women [20], and among Israelis 16.5% overall, 15.1% for women [21].

In this study, egg size (approximately 60–65 g edible weight) and sensory evaluations by volunteer consumers did not differ significantly between control and fortified egg ($P > 0.05$), both being highly acceptable. Feed cost analysis showed an average increase of 0.02 NIS (new Israeli shekels)/egg above 0.54 NIS total feed cost/egg, translating to an increase of 1.5–3.0% in total egg cost, subject to market feed prices.

Discussion

Consumption of one fortified egg, with increases in total n-3 PUFA by 3.8-fold and DHA by 2.4-fold, could make a significant dietary contribution, particularly of n-3 LCPUFA, with DHA content equaling 33.7 and 41.4% of the upper DRI for Americans and Israelis, respectively.

The substantial increase in egg n-3 LCPUFA is in accordance with previous studies, with relatively high final DHA content [8,11,12]. This demonstrated the exceptional effectiveness of the egg for transforming ALA (18:3 n-3 PUFA) to DHA (22:6 n-3 LCPUFA), even in a high n-6 PUFA environment, and without an accompanying increase in n-6 LCPUFA, i.e., AA [Table 1]. In humans, conversion of ALA to DHA can be quite low: 0.05–4.0% [23]. Pre-formed n-3 LCPUFA is particularly important, facing relative n-3 PUFA precursor scarcity [8,9] and enzymatic competition by high intake of n-6 PUFA LA, as n-6 and n-3 undergo transformation via the same enzyme [17].

The highly effective incorporation and transformation of supplemental feed ALA to egg yolk lipids [13] enables fortified egg to become a significant dietary source of DHA; although lower than coldwater marine fatty fish, it does not present the drawback of environmental contaminants [10]. The recommended intake of two 100 g servings/week of fatty fish [6] could provide 350–870 mg/day n-3 PUFA (mostly LCPUFA) [10], while one fortified egg yielded nearly 260 mg total n-3 PUFA, approximately 100 mg as DHA. Where daily consumption of 0.7 (United States) and 0.5 (Israel) regular egg/day contributes only a minor amount of n-3 PUFA, increasing daily consumption to a whole fortified egg could double the current dietary DHA intake in men and women. Young women could attain the most significant benefit, as their current total n-3 PUFA intake is lower and capacity for ALA transformation to DHA is greater [23]. Their requirements for DHA can be much higher, due to the extensive mobilization of DHA during pregnancy and lactation for fetal and infant brain development, risking maternal depletion and negative gestational outcomes [5].

The dietary cholesterol complementary addition of approximately 64 or 107 mg/day, by consuming a whole egg vs. current intake of 0.7 and 0.5 egg/day, may not necessarily be associated with a widespread increase in diet-related cholesterol risk. This is because average and median cholesterol intakes in Americans and Israelis are currently lower than the upper recommended level [18,19], their reported hypercholesterolemia rates are approximately 17.7 and 16.5% [20,21], and dietary cholesterol hyper-responders are presumed to comprise 33.3% of the general population [24]. Interestingly, a recent large nationally representative cohort in the United States showed that consumption of > 6 eggs/week (average of ≈1/day) did not increase cardiovascular disease risk, except for diabetics [25]. Canadian health authorities have recently decided against an upper limit for cholesterol intake.

As previous research has shown a significant positive impact of consumption of n-3 PUFA-fortified eggs, with no noted negative effects [8,14,16], it has been suggested that they may be used as one means of increasing n-3 PUFA consumption to meet current recommendations [8]. The present study suggests that simple and low cost n-3 PUFA egg fortification may yield a significant dietary contribution, especially a high %DRI of DHA, and could be relevant to the United States, Israel, and other western countries that have high total n-6 PUFA and relatively low n-3 PUFA intakes. This could be critical in light of the important benefits of adequate n-3 PUFA (especially n-3 LCPUFA) and low dietary n-6:n-3 PUFA ratio in diabetes, cardiovascular disease, brain development and function, and more [2,5-8].

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