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# n-3 PUFA Fortification of High n-6 PUFA Farmed Tilapia with Linseed Could Significantly Increase Dietary Contribution and Support Nutritional Expectations of Fish

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Farmed fish high in n-6 PUFA may undermine fish nutritional expectations and intake recommendations for n-3 PUFA requirements and exacerbate rather than improve already high n-6/n-3 PUFA diets. Dietary contribution of fish fortification by linseed-based n-3 PUFA was evaluated. Mango tilapia (12 months old) with high n-6 PUFA (21.8 FA%, n-6/n-3 ratio 4.6:1) were fed standard/control ($T_C$) or linseed-supplemented (5%, $T_{5\%}$; 7%, $T_{7\%}$) feed for 61 days regular-growth and 120 days stock-growth (to 650 g). Compared to $T_C$, n-3 PUFA increased in $T_{5\%}$ 46% and $T_{7\%}$ 58%; ALA in $T_{5\%}$ increased 100% and $T_{7\%}$ 167%; EPA+DHA in $T_{5\%}$ increased 14% and $T_{7\%}$ 23% ($p < 0.05$); n-6 PUFA/LCPUFA were unchanged. $T_{7\%}$ EPA+DHA 168 mg/100 g of raw fillet is comparable to current American intake and Dietary Reference Intakes; controlled cooking preserved ≈90% EPA+DHA. n-6/n-3 ratios decreased 16−38% in total PUFA to 2.3:1 and in LCPUFA to 0.61:1. Linseed supplementation could improve tilapia n-3 PUFA/LCPUFA, ameliorating n-3 PUFA scarcity and unexpectedly high fish n-6 PUFA content, potentially making a significant nutritional contribution.

**KEYWORDS: n-3 PUFA; LCPUFA; fish; fortification; linseed**

## INTRODUCTION

n-3 polyunsaturated fatty acids (PUFA) have been suggested to benefit heart disease, metabolic syndrome, rheumatoid arthritis, and other inflammatory conditions, pulmonary disorders, and some psychiatric disorders (*1*−*4*), as well as support of brain function and development, particularly during the perinatal period (*5*, *6*). These have been attributed mostly to n-3 long-chain PUFA (LCPUFA), specifically eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) (*2*), highly concentrated in coldwater marine fish (*7*). Recent research continues to suggest that the typical Western diet may be deficient in n-3 PUFA (*8*), corroborating earlier findings (*7*), with fish being the primary food source (*9*).

High dietary n-6 PUFA and n-6/n-3 PUFA ratios have been suggested to play a role in many Western chronic diseases (*4*, *10*, *11*). American and Israeli diets contain two of the highest n-6 PUFA levels and n-6/n-3 PUFA ratios (15.7 g/day and 9.8−8.7:1, respectively) (*12*, *13*), nearly twice those of the traditional Mediterranean diet (7.8 g/day and 4.3:1, respectively), considered to be a "gold standard" for cardiac disease prevention (*14*).

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A recent position paper by the American Dietetic Association, in collaboration with Dietitians of Canada, emphasizes the food-based approach to attaining recommendations for fatty acids (FA) by including "fatty fish high in n-3 fatty acids" (*15*). This has been translated to a quantitative recommendation of two fish servings/week of approximately 4 oz (100 g cooked ≈ 130 g raw) each (*16, 17*).

Whereas the high n-3 LCPUFA content innate in coldwater marine fish was previously ensured in farmed fish by aquaculture practices of incorporating fish oil into feed, the current scarcity of wild fish (*18, 19*) has led to inconsistent supply, with increased use of high n-6 PUFA feed sources in aquaculture (*20*). This has caused fish to vary by orders of magnitude in n-3 LCPUFA content and for some to have unexpectedly high n-6 PUFA and n-6/n-3 PUFA ratios (*9*).

Tilapia—one of the most popular farmed fish worldwide and most widely farmed in the United States (*9*)—was recently found to have very little n-3 PUFA and one of the highest n-6 PUFA contents and n-6/n-3 LCPUFA ratios among commonly consumed farmed fish, raising concern of its becoming one of the richest sources of n-6 LCPUFA (*9*). It may yield 40-fold higher n-6/n-3 PUFA ratios than coldwater fish, up to 6.0:1 versus 0.10−0.26:1, respectively (*9, 21, 22*).

High levels of n-6 PUFA, especially LCPUFA, have been suggested to enhance inflammatory cascades (*23*) and risk of metabolic/cardiovascular disorders and cancer (*3, 15, 16, 18, 24, 25*) and are suspected to partially underlie low health status in Western countries (*4*), as in the "Israeli paradox" of ill health partially attributed to high n-6 PUFA, versus otherwise good dietary characteristics (*10, 11*). Low dietary n-3 LCPUFA availability may be exacerbated by scarcity in aquaculture and avoidance of the richest marine sources due to contamination (*21*), which may also reduce the net benefits of intake (*7, 26*).

Serum FA profiles of humans have been shown to mirror those of the fish consumed (being the main dietary n-3 PUFA source) and respective fish feeds, and significant reductions of serum triglycerides and inflammatory markers have been noted with the highest n-3 LCPUFA fish (*27*). As an herbivorous species, tilapia could be relevant for vegetal n-3 PUFA fortification, as it has the enzymes required for endogenous production of n-3 LCPUFA (i.e., EPA 20:5 and DHA 22:6) from shorter chain precursor α-linolenic acid (ALA, 18:3 n-3 PUFA); piscivorous fish (i.e., turbot, sea bream) lack this capability and can therefore only concentrate preformed LCPUFA, mostly from algae-consuming prey. As coldwater temperatures increase activity of δ-6-desaturase, the primary enzyme required for n-3 PUFA transformation (*28*), fish grown in warm water regions have an environmental disadvantage in this regard.

Linseed, a land-based n-3 PUFA source rich in ALA [53% of total FA (FA%)], has been found to be nearly 4 times more effective than canola oil (17.5 FA%) in increasing fish n-3 PUFA, reducing n-6/n-3 PUFA ratio (*20*), and increasing their concentrations of ALA and n-3 PUFA anabolites EPA and DHA (i.e., in Nile tilapia, *Oreochromis niloticus*), although much lower than in coldwater marine fish. Reduced deposition of n-6 PUFA, including linoleic acid (LA, 18:2) and arachidonic acid (AA, 20:4 n-6), is highly significant in n-3 PUFA supplementation of high n-6 PUFA fish feed (*29*). Whole linseed may be recommended over linseed oil due to its lower tendency toward oxidation, and the extrusion process was found to neutralize antinutritional factors and increase n-3 PUFA digestibility and metabolism compared to unprocessed seeds (*30*).

Here, we studied the possibility of n-3 PUFA fortification of a popular herbivorous fish—tilapia—with extruded linseed (ELS), a vegetal, land-based 18:3 source. Contents of n-6 and n-3 PUFA in fortified fish were evaluated for their contribution to consumers in conditions of high n-6 PUFA fish feed and human diets.

**Table 1.** Fish Feed Mixture FA Compositions: Control and ELS-Supplemented (5 and 7%)

| feed | control | ELS 5% | ELS 7% |
|---|---|---|---|
| total fat (%) | 5.3 | 5.2 | 5.3 |
| FA (%) | | | |
| 16:0 | 20.07 | 16.51 | 16.59 |
| 16:1 n-9 | 1.64 | 2.19 | 1.19 |
| 18:0 | 4.31 | 4.97 | 4.32 |
| 18:1n-9 | 26.16 | 26.98 | 24.91 |
| 18:2 n-6 | 39.02 | 33.75 | 33.71 |
| 18:3 n-3 | 2.72 | 7.39 | 13.99 |
| 20:1 n-9 | 0.78 | 0.73 | 0.64 |
| 20:4 n-6 | 0.19 | 0.26 | 0.17 |
| 20:4 n-3 | 0.08 | 0.10 | 0.06 |
| 20:5 n-3 | 0.14 | 0.13 | 0.12 |
| 22:5 n-3 | 0.02 | 0.02 | 0.02 |
| 22:6 n-3 | 0.04 | 0.05 | 0.05 |
| SFA | 26.763 | 23.946 | 22.905 |
| MUFA | 29.088 | 30.489 | 27.223 |
| PUFA | 44.148 | 45.565 | 49.873 |
| n-3 PUFA | 3.771 | 8.75 | 14.983 |
| n-6 PUFA | 40.377 | 36.815 | 34.89 |
| n-6/n-3 ratio | 10.707 | 4.2 | 2.329 |

## MATERIALS AND METHODS

**Feeding Groups.** Commercial mango tilapia (*Sarotherodon galilaeus galilaeus*; Beit Shean Livestock, Israel), aged 12 months at study start, were fed ad libitum either standard (control) feed ($T_C$; total $n = 600$, divided into 4 replicate tanks of 150 fish each) or standard feed supplemented with ELS at concentrations of 5% ($T_{5\%}$; $n = 600$, $4 \times 150$) or 7% ($T_{7\%}$; $n = 600$, $4 \times 150$). Tanks were designated by feeding group. Fish were cared for and used professionally and humanely, maintained in a controlled environment appropriate to the species, with limited stress.

**Growth Conditions.** Fish were grown in commercial tanks, in a commercial setting. An initial experimental "regular" growth feeding cycle of 61days was followed by 120 days of "stock" growth with reduced feed amounts, initially for attaining market size and then for maintaining market-acceptable weight of approximately 620−650 g (to 180 days total), while limiting overcrowding in tanks. Tank temperatures were $27 \pm 0.5$ and $28 \pm 0.5$ °C, respectively. Conditions were identical among all replicates.

**Fish Feeds.** Feed provided was the sole source of nutrition for the fish, with no extraneous sources available in the growing tanks. Feed formulations were prepared by Shivuk Raanana (Israel), based on meals of soy 28.0%, fish 8%, wheat 12%, corn 9.5%, feather 8.5%, meat 8%, and sunflower-based mix 6%, plus a lysine supplement 0.15%. Formulations differed principally by the addition of ELS ($T_C$ 0% vs $T_{5\%}$ 5% and $T_{7\%}$ 7%), proportionally offset by bran meal ($T_C$ 20%, $T_{5\%}$ 15%, $T_{7\%}$ 13%). Feed macronutrient contents were similar in all study groups, averaging protein 35%, ash 6%, fiber 4%, and fat 4%, differing in FA composition as detailed in **Table 1**. ELS mix of 70% linseed and 30% wheat bran contains the following: crude protein, 20%; available carbohydrates, 14.5%; nondietary fibers, 22.4%; ash, 4%; lignin, 4.8%; and fat, 28%, with n-6 PUFA 4.7% and n-3 PUFA 15.7%; oil content of ELS yields a fat composition of ALA 57%, LA 16%, oleic acid 18%, and saturated fatty acids (SFA) 9% (Valorex Technical Data Sheet EV 958-04).

**Sample Selection.** At each time interval, fish were removed for analysis randomly from each of four designated growth tanks. Following descaling and gutting, a strip of flesh ("fillet", representing edible portion) between the pelvic and dorsal fins was removed for processing. Four batched replicates of four 100-g fillets per test group were pooled at each interval (0, 60, 180 days) for analysis.

**Fish Cooking.** Cooking by two popular methods (baking, frying) was applied to $T_C$, $T_{5\%}$, and $T_{7\%}$ sample fillets to assess relative heat

**Table 2.** Tilapia Body Weight, Biomass Gain, Feed Conversion Rate (FCR), and Specific Growth Rate (SGR) for the Experimental Regular-Growth (0−61 Days) and Stock-Growth (61−180 Days) Periods for Control ($T_C$) and ELS (5, 7%; $T_{5\%}$, $T_{7\%}$) Supplemented Fish

| | treatment | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | $T_C$ | | | $T_{5\%}$ | | | $T_{7\%}$ | | |
| measure | 0 days | 61 days | 180 days | 0 days | 61 days | 180 days | 0 days | 61 days | 180 days |
| weight (g) | 408.2 ± 24.0 | 480.5 ± 6.0 | 640.1 ± 11.7 | 400.2 ± 18.0 | 490.4 ± 14.7 | 650.5 ± 14.8 | 375.6 ± 28.0 | 452.2 ± 22.0 | 623.0 ± 20.5 |
| biomass (kg) | 12.0 ± 0.8 | 13.1 ± 0.3 | 15.4 ± 0.6 | 12.2 ± 0.6 | 13.2 ± 0.4 | 15.3 ± 0.6 | 11.2 ± 0.8 | 12.2 ± 0.6 | 14.3 ± 0.8 |
| FCR | | 3.6 ± 1.2 | 3.0 ± 1.2 | | 3.2 ± 1.1 | 2.6 ± 1.6 | | 3.3 ± 0.8 | 3.3 ± 0.5 |
| SGR (%/day) | | 0.30 ± 0.04 | 0.40 ± 0.02 | | 0.32 ± 0.08 | 0.40 ± 0.05 | | 0.33 ± 0.05 | 0.39 ± 0.01 |

**Table 3.** Tilapia Fillet Fat and FA% at Experimental 0, 61, and 180 Days of Control ($T_C$) and ELS (5, 7%; $T_{5\%}$, $T_{7\%}$) Supplemented Feed

| | feed group | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | control ($T_C$) | | | 5% ELS ($T_{5\%}$) | | | 7% ELS ($T_{7\%}$) | | |
| | 0 days[a] | 61 days | 180 days | 0 days[a] | 61 days[b] | 180 days[b] | 0 days[a] | 61 days[b] | 180 days[b] |
| fat (wt %) | 4.7 ± 0.5 | 6.3 ± 0.7 | 7.1 ± 1.1 | 4.7 ± 0.4 | 5.9 ± 0.6 | 7.9 ± 1.1 | 5.1 ± 0.4 | 5.6 ± 0.6 | 7.3 ± 0.9 |
| total SFA | 31.7 ± 0.7 | 33.5 ± 0.6 | 31.9 ± 0.7 | 31.5 ± 0.7 | 32.7 ± 0.3 | 29.9 ± 0.7 | 31.5 ± 0.5 | 31.9 ± 0.9 | 30.5 ± 0.6 |
| total MUFA | 40.9 ± 0.4 | 43.0 ± 0.2 | 43.0 ± 0.6 | 41.3 ± 0.5 | 42.4 ± 0.4 | 43.7 ± 0.4 | 42.2 ± 0.4 | 41.9 ± 0.3 | 41.8 ± 0.7 |
| total PUFA | 27.4 ± 0.4 | 23.5 ± 0.5 | 25.1 ± 0.7 | 27.2 ± 0.6 | 24.9 ± 0.5b | 26.5 ± 1.0a | 26.0 ± 0.5 | 26.1 ± 0.6b | 27.7 ± 0.4a |
| total n-6 PUFA[c] | 21.8 ± 0.5 | 19.4 ± 0.4 | 19.6 ± 0.4 | 21.8 ± 1.0 | 18.6 ± 0.4a | 19.1 ± 1.3 | 21.0 ± 0.2 | 19.0 ± 0.5 | 19.2 ± 1.3 |
| LA (18:2) | 17.9 ± 0.2 | 16.0 ± 0.3 | 15.9 ± 0.6 | 17.7 ± 0.4 | 15.4 ± 0.3a | 15.5 ± 0.5 | 17.4 ± 0.4 | 15.5 ± 0.3 | 15.5 ± 0.2 |
| AA (20:4) | 1.1 ± 0.1 | 1.0 ± 0.1 | 1.1 ± 0.1 | 1.2 ± 0.1 | 1.0 ± 0.0 | 1.0 ± 0.1 | 1.0 ± 0.1 | 1.1 ± 0.1 | 1.1 ± 0.1 |
| total n-6 LCPUFA[d] | 3.1 ± 0.2 | 2.7 ± 0.1 | 3.0 ± 0.2 | 3.2 ± 0.3 | 2.6 ± 0.1 | 2.9 ± 0.4 | 2.8 ± 0.2 | 2.9 ± 0.2 | 3.0 ± 0.3 |
| total n-3 PUFA[e] | 5.5 ± 0.2 | 4.2 ± 0.3 | 5.3 ± 0.1 | 5.2 ± 0.2 | 6.4 ± 0.3b | 7.2 ± 0.4a | 4.9 ± 0.1 | 7.0 ± 0.4b | 8.4 ± 0.5b |
| ALA (18:3) | 1.6 ± 0.2 | 1.2 ± 0.1 | 1.2 ± 0.1 | 1.3 ± 0.1 | 2.8 ± 0.4b | 2.4 ± 0.1b | 1.3 ± 0.1 | 3.1 ± 0.2c | 3.2 ± 0.3c |
| EPA (20:5) | 0.4 ± 0.0 | 0.3 ± 0.0 | 0.3 ± 0.0 | 0.4 ± 0.0 | 0.3 ± 0.0 | 0.4 ± 0.0 | 0.4 ± 0.0 | 0.3 ± 0.0 | 0.4 ± 0.0 |
| DHA (22:6) | 2.1 ± 0.1 | 1.8 ± 0.1 | 1.9 ± 0.1 | 2.2 ± 0.2 | 2.0 ± 0.1 | 2.1 ± 0.1 | 1.9 ± 0.1 | 2.3 ± 0.2a | 2.3 ± 0.1a |
| total n-3 LCPUFA[f] | 3.6 ± 0.3 | 2.9 ± 0.2 | 3.8 ± 0.1 | 3.6 ± 0.3 | 3.5 ± 0.2a | 4.4 ± 0.4 | 3.3 ± 0.2 | 4.0 ± 0.4a | 4.9 ± 0.3a |
| n-6/n-3 PUFA ratio | 4.0 ± 0.2 | 4.6 ± 0.3 | 3.7 ± 0.4 | 4.2 ± 0.2 | 2.9 ± 0.1b | 2.7 ± 0.1b | 4.3 ± 0.2 | 2.7 ± 0.1b | 2.3 ± 0.1a |
| n-6/n-3 LCPUFA ratio | 0.86 ± 0.1 | 0.93 ± 0.1 | 0.79 ± 0.1 | 0.90 ± 0.1 | 0.72 ± 0.0a | 0.66 ± 0.03 | 0.84 ± 0.1 | 0.75 ± 0.0a | 0.61 ± 0.1b |

[a] Mean values at 0 days were attained by analysis of blended samples; standard deviations are not available. [b] a, $p < 0.05$; b, $p < 0.005$; c, $p < 0.0005$ vs control. [c] Total n-6 PUFA include the following FA: 18:2, 18:3, 20:2, 20:3, 20:4, 22:2, 22:3, 22:4, 22:5. [d] Total n-6 LCPUFA include the following FA: 20:2, 20:3, 20:4, 22:2, 22:3, 22:4, 22:5. [e] Total n-3 PUFA include the following FA: 18:3, 18:4, 20:3, 20:4, 20:5, 21:5, 22:3, 22:5, 22:6. [f] Total n-3 LCPUFA include the following FA: 20:3, 20:4, 20:5, 21:5, 22:3, 22:5, 22:6.

and oxidation-related PUFA losses in control and fortified fish. Fillets of $T_C$, $T_{5\%}$,, and $T_{7\%}$ at 61days were either oven-baked (no added fat) for 30 min at 180 °C or pan-fried for 16 min total, 8 min each side, in preheated (for 1.5 min) canola oil (1 tablespoon, 14 g), which contains 1.3 g of n-3 and 2.8 g of n-6 PUFA (Ben Gurion University Israeli Food Nutrient Database).

**Fat Composition Analysis.** Fillet fat percent was evaluated according to the Folch method (*31*), employing cold extraction with a 2:1 chloroform/methanol mixture. FA profiles (percentage of total FA) were analyzed in raw and cooked fish by fat extraction (Directive European 98/64/CE process B), methylation with 0.5 mol/L sodium hydroxide and 12−15% boron trifluoride according to norme Français (NF) T-60 233, and gas chromatographic separation and quantification per NF V03-772 (*30*). An internal standard (C13:0) was used according to AOAC Official Method 996.06 for fat analysis in foods.

**Statistical Analysis.** Data were analyzed as one-way ANOVA, with Duncan's multiple-range means separation tests.

**Dietary Contribution.** n-3 and n-6 PUFA contents of 100 g of raw fillet of $T_{7\%}$ were compared to current daily FA intakes of American and Israeli adults >19 years (*12*, *13*) and to Dietary Reference Intakes (DRI). On the basis of relative daily intake ranges, as recommended by the Institute of Medicine (IOM) for ALA [0.6−1.2% of total caloric intake (kcal %)] and EPA+DHA (0.06−0.12 kcal %), the minimum daily amounts were used here as minimum reference DRI: ALA, 1.6 g for men, 1.1−1.4 g for women (1.1 g nonpregnant/lactating, 1.3 g pregnant, 1.4 g lactating); EPA+DHA, 160 mg for men, 110−140 mg for women. The DRI for LA are 14−17 g for men (requirement decreasing with age) and 11−13 g for women (11−12 g nonpregnant/lactating, 13 g pregnant/lactating).

**Cost Analysis.** Calculations were conducted by exchanging respective costs of 5 and 7% ELS supplement with standard feed components (Alonim Ltd., Israel).

## RESULTS

Feed supplementation with ELS did not affect feed intake, feed conversion rate (FCR), specific growth rate (SGR), body weight (**Table 2**), or fat percent (**Table 3**) compared to the control group. Daily feed intake averaged 1.6% of body weight (7 g/day) during the first experimental "regular-growth" period of 0−61 days, reduced to 0.5% of body weight (2.5 g/day) during the second "stock-growth" period of 61−180 days, in all groups. Correspondingly, average weight gains were faster during the regular- versus stock-growth period, averaging 1.5 versus 1.3 g/day, respectively, to market weight of 620−650 g.

Throughout the study, fish flesh total PUFA, MUFA, and SFA FA% fluctuated within ±8% (NS) (**Table 3**).

Body fat n-3 PUFA FA% increased significantly with linseed concentration (ELS%) and feeding time: overall (0−180 days) total n-3 PUFA increased in $T_{5\%}$ and $T_{7\%}$ by 46 and 58%, respectively ($p < 0.05$), with no increase in $T_C$ (**Table 3**); ALA in $T_{5\%}$ and $T_{7\%}$ increased 158 and 167% ($p < 0.05$); EPA was not significantly changed; DHA increased 20% in both $T_{5\%}$ and $T_{7\%}$ ($p < 0.05$); and total n-3 LCPUFA (including 20:3, 20:4, 20:5, 21:5, 22:3, 22:4, 22:5, 22:6) increased in $T_{5\%}$ by 15% and in $T_{7\%}$ by 30%.

Raw fillets (100 g) of $T_{5\%}$ and $T_{7\%}$ (180 days) contained 480 and 520 mg of total n-3 PUFA, respectively, versus 320 mg in $T_C$; ALA contents were 160 and 200 mg versus 72 mg, respectively, and EPA+DHA contents were 168 versus 132 mg ($p < 0.05$). Fortified fish yields attained >10% of the minimum DRI for ALA and >100% for EPA+DHA.

**Table 4.** n-6 and n-3 PUFA Amounts per Portion (Grams per 100 g) of Raw versus Cooked Tilapia Fillet (Experimental 61 Days), Control ($T_C$), and Fortified (ELS 5, 7%; $T_{5\%}$, $T_{7\%}$)

| | feed group | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | $T_C$ | | | $T_{5\%}$ | | | $T_{7\%}$ | | |
| | raw | baked | fried | raw | baked | fried | raw | baked | fried |
| total n-6 PUFA | 1.978 | 1.885 | 1.830 | 1.748 | 1.646 | 1.841 | 1.929 | 1.862 | 1.953 |
| LA (18:2) | 1.632 | 1.597 | 1.540 | 1.448 | 1.399 | 1.554 | 1.566 | 1.607 | 1.670 |
| AA (20:4) | 0.102 | 0.082 | 0.070 | 0.094 | 0.062 | 0.079 | 0.111 | 0.078 | 0.084 |
| total n-6 LCPUFA | 0.275 | 0.237 | 0.220 | 0.235 | 0.202 | 0.218 | 0.293 | 0.245 | 0.221 |
| total n-3 PUFA | 0.428 | 0.391 | 0.360 | 0.602 | 0.449 | 0.545 | 0.707 | 0.657 | 0.819 |
| ALA (18:3) | 0.122 | 0.124 | 0.170 | 0.263 | 0.202 | 0.257 | 0.313 | 0.304 | 0.441 |
| EPA (20:5) | 0.031 | 0.021 | 0.020 | 0.028 | 0.026 | 0.030 | 0.030 | 0.029 | 0.042 |
| DHA (22:6) | 0.184 | 0.165 | 0.160 | 0.188 | 0.132 | 0.149 | 0.232 | 0.196 | 0.200 |
| total n-3 LCPUFA | 0.296 | 0.268 | 0.260 | 0.329 | 0.246 | 0.277 | 0.394 | 0.343 | 0.378 |
| n-6/n-3 PUFA | 4.6 ± 0.2 | 4.9 ± 0.3 | 5.0 ± 0.1 | 2.9 ± 0.2 | 3.7 ± 0.3 | 3.4 ± 0.4 | 2.7 ± 0.2 | 2.9 ± 0.3 | 2.4 ± 0.2 |
| n-6/n-3 LCPUFA | 0.94 ± 0.0 | 0.85 ± 0.0 | 0.85 ± 0.0 | 0.72 ± 0.0 | 0.82 ± 0.0 | 0.79 ± 0.0 | 0.75 ± 0.0 | 0.71 ± 0.0 | 0.58 ± 0.0 |

Current intakes of total n-3 PUFA among Americans and Israelis (1.9−2.1 g/day in men, 1.4 g/day in women) meet the minimum DRI, but EPA+DHA in Americans (137 mg/day in men, 97 mg/day in women) reach only 85% for men, 88% for nonpregnant/lactating women, and <75% during pregnancy and lactation (*12*, *13*) (Israeli data for EPA+DHA are not available). Current intakes would be substantially increased with consumption of fortified fish fillet, 100 g/day raising total n-3 PUFA by >25% and EPA+DHA to >120%.

Baseline (0 days) total n-6 PUFA FA% were 21.0−21.8%, with LA 17.4−17.9% and AA 1.0−1.2%. Although 180 d total n-6 PUFA and LA declined approximately 10% in both control and fortified fish, AA remained relatively unchanged (**Table 3**). Final LA yield was 1 g/100 g of fillet in all groups (<10% DRI); AA yield was 67 mg/100 g (no established DRI). Total n-6/n-3 PUFA ratios declined in $T_{5\%}$−$T_{7\%}$ 27−38% compared to $T_C$, and n-6/n-3 LCPUFA ratios declined 16−23% ($p < 0.05$) (**Table 3**).

With controlled baking and frying (**Table 4**), in $T_C$ total n-3 PUFA decreased by 9 and 14%, respectively, total n-3 LCPUFA FA% decreased by approximately 10%, EPA remained unchanged, and DHA declined by 11%. DHA declined also in $T_{7\%}$ 13−17% (baking−frying); however, with frying total n-3 PUFA increased in $T_{7\%}$ by 11%, and ALA FA% increased in $T_C$ by 42% and in $T_{7\%}$ 35.5%. Total n-6 PUFA changed in all groups ±4−6%; n-6 LCPUFA losses were greatest, down in $T_C$ by 15−18% and in $T_{7\%}$ by 14−28% (baking−frying), with AA declining in $T_C$ by 30% and in $T_{7\%}$ by 27% (both baking and frying). SFA remained unchanged, whereas MUFA increased nonsignificantly.

Financial analysis revealed that the extra feed cost with the addition of 5−7% ELS may vary according to market price. At the time of the present study, feed costs were approximately 3.0−4.5% greater than standard (control).

## DISCUSSION

Contrary to expectations that all fish will contain significant amounts of n-3 PUFA and have a low n-6/n-3 PUFA ratio (*21*, *22*), warmwater farmed mango tilapia with high n-6 PUFA content (total n-6 21.8 FA%) and n-6/n-3 PUFA ratio (4.7:1) and low n-3 LCPUFA (3.6 FA%) could exacerbate rather than ameliorate high dietary n-6 PUFA and n-6/n-3 PUFA ratios. Moreover, this could undermine reliance on fish as a major source of n-3 LCPUFA and existing fish intake recommendations of two 100 g servings/week. Given that tilapia is the fastest growing fish in aquaculture and consumption and has among the highest contents of n-6 PUFA, n-6 LCPUFA, and n-6/n-3 LCPUFA ratio (*9*), the scope of the problem and health implications are likely to increase as well.

The present study showed that simple and low-cost fortification with a linseed preparation (ELS) could partially support expectations of fish to be a consistent source of n-3 PUFA and make a dietary contribution to their requirements versus high n-6 PUFA Western diets, as in America and Israel. Tilapia fillets following fortification with 7% ELS for 180 days yielded 168 mg total EPA+DHA per 100 g of raw fish, approximately 12% of the benchmark contribution of wild salmon (*21*). Two weekly $T_{7\%}$ servings (100 g cooked ≈ 130 g raw) as recommended (*16*, *17*) would contribute significant EPA+DHA (55 mg/day, 33−50% DRI) and, to a lesser degree, ALA (65 mg/day, 4% DRI), with only a minor LA contribution (370 mg, 2−3% DRI). Substituting fortified for regular farmed tilapia could raise current American EPA+DHA intake to attain the minimum DRI, where it currently averages 87% (<75% for pregnant and lactating women).

Body fat content matched the range of other commercial tilapia (*24*, *32*); initially high $T_C$ n-6 PUFA FA% values were similar to those of Australian warmwater wild fish (*20*, *22*) and to Nile tilapia in Thailand given high corn oil feed (*29*), but AA content was half that observed in a recent evaluation of farmed tilapia samples from around the world (*9*).

Despite low endogenous n-3 LCPUFA production during the regular-growth supplementation period (0−61 days), which is consistent with results in farmed tilapia (*29*) and Atlantic salmon fed canola (*27*), a gradual cumulative increase through the later stock-growth period (61−180 days) added nearly 50% of final values in $T_{5\%}$ and $T_{7\%}$. Stock growth in certain regions is used to accommodate market demands regarding amounts and sizes versus seasonal fluctuations in temperatures and growth rates. In the present study, all groups appeared to have equally slow growth with high values for FCR, as compared to faster growth values found in other batches of tilapia later raised in the same tanks, although all had similar n-3 PUFA increases. Overall, n-3 PUFA supplementation for 6 months, one-third the total growing period of 18 months and similar to the time frame of high fish oil "finishing diets" (*20*), could be used to moderately increase n-3 PUFA with linseed in tilapia. The small increases in n-3 LCPUFA observed with fortification may support the known innate capacity of herbivorous fish such as tilapia for n-3 PUFA transformation (from 18:3 to 22:6) and accretion, although the high feed n-6 PUFA and warmwater conditions may have minimized the transformation potential (*28*).

Although uncontrolled cooking conditions may lead to significant n-3 PUFA oxidation loss (*33*), the controlled

procedures in the present study showed that total n-3 PUFA and n-3 LCPUFA could be greatly preserved in baking and frying. Unexpectedly, n-3 PUFA was more preserved than n-6 LCPUFA, and ALA was increased in frying more than oleic acid (18:1 n-9 MUFA), despite a higher proportion of oleic acid in the canola frying oil and its lower susceptibility to oxidation.

Beyond the general contribution to heart health and reduced triglycerides and inflammatory markers seen with high n-3 LCPUFA fish (*27*), women could benefit more than men from fish fortification: first, they currently tend to consume lower amounts of n-3 PUFA, including n-3 LCPUFA (*12*, *13*); second, their n-3 PUFA requirements and capacity for transforming ALA to DHA are much higher than men's and are further heightened during pregnancy and lactation (*34*), during which maternal body reserves may be depleted and cause secondary deficiencies (*5*, *6*). Moreover, women are more likely to avoid marine fatty fish, as they are more cautious about contamination of fish during their childbearing years (*35*).

Mango tilapia growing semiwild (restricted fish population) in the Sea of Galilee, a freshwater lake, were found to contain n-3 LCPUFA levels 265% those of $T_{7\%}$, largely from wild algae growing in the lake (Shapira et al., 2008, unpublished findings). These findings suggest that a natural algae food supply, an important source of n-3 LCPUFA for wild coldwater marine fish, may be applicable even in a warmwater/freshwater aquaculture environment (*36*).

The feasibility of increasing farmed fish total n-3 PUFA sharply and n-3 LCPUFA moderately, reducing n-6/n-3 PUFA ratios, and limiting transformation of LA to higher risk AA presents an important opportunity for health-oriented aquaculture and food production. Although final n-3 LCPUFA content is still far lower compared to coldwater marine fatty fish, efficient land-based n-3 PUFA fortification may yield significant potential for adapting farmed fish to provide needs for n-3 LCPUFA in high n-6 PUFA human diets. This is especially important in light of increasing food costs and environmental awareness, which will require attaining more of our needs with fewer and more sustainable resources.

## ABBREVIATIONS USED

AA, arachidonic acid; ALA, α-linolenic acid; ANOVA, analysis of variance; CDC, Centers for Disease Control; DHA, docohexaenoic acid; DRI, dietary reference intake(s); ELS, extruded linseed; EPA, eicosahexaenoic acid; FA, fatty acid (s); ICDC, Israel Centers for Disease Control; LA, linoleic acid; LCPUFA, long-chain polyunsaturated fatty acid(s); MUFA, monounsaturated fatty acid(s); n-, *ω*-; NF, norme Français; PUFA, polyunsaturated fatty acid(s); SFA, saturated fatty acid(s); T, tilapia.

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