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# Creating ω-3 Fatty-Acid-Enriched Chicken Using Defatted Green Microalgal Biomass

Stephanie K. Gatrell, JongGun Kim, Theodore J. Derksen, Eleanore V. O'Neil, and Xin Gen Lei*

Department of Animal Science, Cornell University, Ithaca, New York 14853, United States

Supporting Information

**ABSTRACT:** This study was to create an ω-3 (n-3) fatty-acid-enriched chicken product using defatted green microalgae (DGA, *Nannochloropsis oceanica*) biomass out of biofuel research. Hatching Ross broiler chicks were fed a corn−soybean meal diet containing 0 (control), 2, 4, 8, or 16% DGA for 6 weeks ($n$ = 6 cages/diet). The DGA inclusion resulted in a linear ($p < 0.001$) increase in total n-3 fatty acids, eicosapentaenoic acid (EPA), and docosahexaenoic acid (DHA) in plasma, liver, breast, and thigh at weeks 3 and 6. The increase in the breast EPA + DHA by the 16% DGA diet reached 60-fold ($p < 0.0001$) over the control. The 8 and 4% DGA diets elevated ($p < 0.05$) liver mRNA levels of Δ-9 (88%) and Δ-6 (96) desaturases. In conclusion, 8−16% of the DGA can be added in diets for broilers to produce a n-3 fatty-acid-enriched chicken meat.

**KEYWORDS:** *broiler chicken, defatted microalgal biomass, docosahexaenoic acid, eicosapentaenoic acid, gene expression*

## INTRODUCTION

Consuming diets high in long-chain polyunsaturated fatty acids (PUFAs), in particular ω-3 (n-3) fatty acids, has been linked to a decreased risk of cardiovascular disease, diabetes, arthritis, and cancer.[1−5] However, current Western diets contain an unbalanced ratio of the "pro-inflammatory" ω-6 (n-6) and "anti-inflammatory" n-3 PUFAs. These diets have an average n-6/n-3 fatty acid ratio of 20−30:1, as opposed to the traditional range of 1−2:1.[6] Increasing public awareness of the health benefits of n-3 fatty acids has led researchers attempting to improve the fatty acid profile of commonly consumed animal products. Because the average American consumes approximately 40 kg of chicken annually,[7] this type of meat is a promising candidate for n-3 fatty acid enrichment.

Fatty acid profiles of chicken breast, thigh, and skin are well-known to be highly dependent upon dietary fatty acid composition.[8] Previously, marine sources, mainly fish oil and fish meal,[9−11] were used to enhance n-3 fatty acid incorporation into poultry meat. However, recent increases in the cost of and demand for fishmeal have led to the investigation of alternative n-3 fatty-acid-rich sources for a more sustainable industry. Indeed, dietary inclusion of plant-based α-linolenic acid (ALA)-rich flaxseed[12−14] or canola oil[15] could elevate the n-3 fatty acid content of broiler meat. However, that enhancement resulted from an increased deposition of ALA and not the longer chain docosahexaenoic acid (DHA) or eicosapentaenoic acid (EPA). Metabolically, ALA needs to be converted to DHA and/or EPA to be functionally meaningful in the human body. However, the efficiency of that conversion *in vivo* seems to be limited.[16]

Microalgae contain a superior fatty acid profile with high abundance of EPA and/or DHA to traditional animal feed protein supplements.[17−19] They also contain relatively high amounts of crude protein,[20] essential amino acids,[21] and carotenoids.[22] Supplementation of microalgae to poultry diets has been shown to improve the overall n-3 fatty acid status in egg yolk[17,23] and breast muscle.[16] Our laboratory has been specifically interested in defatted microalgal biomass, the byproduct of biofuel research, as a partial replacement of conventional poultry feed ingredients. In broiler chicks, we have demonstrated that moderate levels of defatted microalgal biomass inclusion exhibited no adverse effects on growth performance or health.[24] However, whether the defatted microalgae, with the residual fatty acids left in the biomass, could enhance the deposition of n-3 fatty acids in the tissues of chicks were not explored.

Consuming diets high in PUFAs ultimately enriches cell membranes in these fatty acids, subsequently altering signaling molecules involved in carbohydrate and lipid metabolism.[25] Enzymes involved in *de novo* fatty acid synthesis, such as malic enzyme (ME) and fatty acid synthase (FASN), are known to be affected by dietary manipulation and feeding status.[26−29] Furthermore, desaturase enzymes that introduce double bonds into fatty acids, including Δ-6 and Δ-9 desaturases, are also affected by the nutritional status.[30,31] Thus, it is fascinating to determine how the defatted microalgal biomass affects the expression of these genes and how that effect is related to the enrichment of n-3 fatty acids in the chick tissues.

Therefore, the objectives of this experiment were (1) to determine the feasibility in producing a n-3 fatty-acid-enriched chicken by feeding broiler chicks increasing levels of a defatted green microalgal biomass (DGA, *Nannochloropsis oceanica*, Cellana, Kailua-Kona, HI) and (2) to reveal the biomass-mediated lipid metabolism gene expression mechanism related to the enrichment of n-3 fatty acids in the tissues of chicks.

## MATERIALS AND METHODS

**Animals, Diets, and Management.** All protocols of this experiment were approved by the Institutional Animal Care and Use Committee (IACUC) of Cornell University (IACUC approval

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**Table 1. Fatty Acid Compositions of DGA and Experimental Diets**

| | | starter diet (weeks 0−3) | | | | | grower diet (weeks 4−6) | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | DGA (%) | | | | | DGA (%) | | | | |
| item | DGA | 0 | 2 | 4 | 8 | 16 | 0 | 2 | 4 | 8 | 16 |
| $C_{14:0}$ | 6.89 | 0.0 | 0.1 | 0.3 | 0.5 | 1.0 | 0.1 | 0.2 | 0.2 | 0.5 | 0.9 |
| $C_{16:0}$ | 27.1 | 12.2 | 12.1 | 12.5 | 13.0 | 14.2 | 12.0 | 12.6 | 12.5 | 13.2 | 13.9 |
| $C_{16:1}$ | 27.3 | 0.1 | 0.5 | 0.9 | 1.8 | 3.8 | 0.1 | 0.6 | 0.9 | 1.9 | 3.5 |
| $C_{18:0}$ | 0.56 | 2.2 | 2.1 | 2.1 | 2.0 | 1.9 | 2.1 | 2.1 | 2.2 | 2.0 | 1.9 |
| $C_{18:1n-9}$ | 13.4 | 26.2 | 26.4 | 25.9 | 25.2 | 25.0 | 25.9 | 25.4 | 26.5 | 25.8 | 24.8 |
| $C_{18:2n-6}$ | 2.09 | 56.4 | 55.8 | 55.2 | 53.8 | 49.3 | 57.2 | 56.4 | 54.6 | 52.9 | 50.5 |
| $C_{18:3n-3}$ | 0.00 | 1.7 | 1.5 | 1.5 | 1.4 | 1.4 | 1.6 | 1.6 | 1.5 | 1.4 | 1.3 |
| $C_{18:3n-6}$ | 0.89 | 0.1 | 0.1 | 0.1 | 0.1 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| $C_{20:0}$ | 0.00 | 0.4 | 0.4 | 0.4 | 0.4 | 0.3 | 0.4 | 0.4 | 0.4 | 0.4 | 0.3 |
| $C_{20:1n-9}$ | 0.00 | 0.3 | 0.3 | 0.3 | 0.3 | 0.2 | 0.3 | 0.3 | 0.3 | 0.2 | 0.2 |
| $C_{20:4n-6}$ | 4.50 | 0.0 | 0.1 | 0.2 | 0.4 | 0.8 | 0.0 | 0.1 | 0.2 | 0.4 | 0.7 |
| $C_{20:5n-3}$ | 16.5 | 0.0 | 0.2 | 0.4 | 0.8 | 1.7 | 0.0 | 0.2 | 0.3 | 0.9 | 1.6 |
| $C_{22:0}$ | 0.00 | 0.2 | 0.2 | 0.1 | 0.1 | 0.1 | 0.2 | 0.2 | 0.2 | 0.2 | 0.1 |
| $C_{22:6n-3}$ | 0.00 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| $C_{24:0}$ | 0.00 | 0.2 | 0.2 | 0.2 | 0.2 | 0.2 | 0.2 | 0.2 | 0.2 | 0.2 | 0.2 |
| total | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 |
| SFA | 35.4 | 15.2 | 15.1 | 15.6 | 16.3 | 17.7 | 15.0 | 15.5 | 15.6 | 16.4 | 17.3 |
| MUFA | 40.7 | 26.6 | 27.1 | 27.1 | 27.3 | 29.1 | 26.2 | 26.2 | 27.6 | 28.0 | 28.5 |
| PUFA | 24.0 | 58.2 | 57.8 | 57.4 | 56.4 | 53.1 | 58.8 | 58.3 | 56.8 | 55.5 | 54.1 |
| n-3 | 16.5 | 1.7 | 1.7 | 1.9 | 2.2 | 3.1 | 1.6 | 1.8 | 1.9 | 2.2 | 2.9 |
| n-6 | 7.47 | 56.4 | 56.0 | 55.4 | 54.2 | 50.1 | 57.2 | 56.5 | 54.9 | 53.3 | 51.3 |
| n-6/n-3 | 0.45 | 33.2 | 32.9 | 29.2 | 24.6 | 16.2 | 35.8 | 31.4 | 28.9 | 24.2 | 17.7 |

number 2010-0106). Male hatchling Ross broiler chicks were obtained from a commercial hatchery and housed in a temperature-controlled unit at the Cornell University Poultry Research Farm. The broiler chicks were housed in thermostatically controlled cage batteries for the first 3 weeks (6 chicks per cage); and 4 chicks per cage (2 killed for sample collection at week 3) were then transferred to grower cages at room temperature from weeks 3−6. Chicks had free access to feed and water and received a lighting schedule of 22 h light and 2 h dark. Birds were fed one of five diets ($n$ = 6 cages/diet) containing 0 (control), 2, 4, 8, or 16% DGA, on an "as-is" basis, replacing a mixture of corn and soybean meal. Nutrient composition of DGA is shown in Table S1. Starter (weeks 0−3; Table S2) and grower (weeks 3−6; Table S3) diets were formulated to be isoenergetic and to meet the requirements for all essential nutrients for each phase of growth.[32] The fatty acid profiles of each starter and grower diet are given in Table 1. At weeks 3 and 6, 2 birds per cage were euthanized via asphyxiation with $CO_2$ and blood was drawn from heart puncture using heparinized needles. After blood was kept on ice and centrifuged at 3000$g$ for 15 min, plasma was stored at −20 °C until analysis. Liver, breast muscle, and legs were removed, and a portion of each was snap-frozen in liquid nitrogen and stored at −80 °C until analysis. Whole skinless breast and legs were sealed in plastic bags and frozen for lipid analysis.

**Fatty Acid Extraction.** For fatty acid extraction, experimental diets (~100 g) were ground to a fine powder. Tissue samples were taken from the liver, the core of the whole breast (pectoralis major), and thigh (bicep femoris). Total lipids from the diet (100 mg), plasma (100 μL), and tissue samples (1 g) were extracted, with some modifications, according to Folsh et al.,[33] and were methylated with methanolic KOH according to Ichihara et al.,[34] using tridecanoic acid (Sigma-Aldrich Co., St. Louis, MO) as an internal standard. Each fatty acid was identified by comparing its retention time and peak area to the individual fatty acid methyl ester standards (Sigma-Aldrich Co., St. Louis, MO). Methyl esters of fatty acids were analyzed using a gas chromatography instrument (Agilent 6890N, Agilent Technologies, Santa Clara, CA) fitted with a flame ionization detector and used a fused-silica capillary column coated with CP-SIL 88 (100 m × 0.25 mm inner diameter, 0.2 mm film thickness, Varian, Inc., Lake Forest, CA). The oven temperature was programmed to be held for 4 min at 140 °C, increased by 4 °C/min to 220 °C, and then held for 5 min. Carrier gas was $N_2$ with a constant flow rate of 2 mL/s; the injector temperature was 230 °C; and the detector temperature was 280 °C. The fatty acid content is expressed as either a weight percentage of the sum of all of the fatty acids analyzed or calculated as milligrams of fatty acid per 100 g of wet tissue.

**Gene Expression.** Real-time reverse transcription polymerase chain reaction (RT-PCR) was performed on the snap-frozen liver samples to estimate the abundance of mRNA using β-actin as a reference gene. Target genes included ME, FASN, and Δ-6 and Δ-9 desaturase. After the RNA was isolated and its quality was verified by agarose gel and spectrometry ($A_{260}/A_{280}$), it was transcribed using a commercially available kit (Applied Biosystems, Grand Island, NY). The resulting cDNA (0.3 μg) was added to a 10 μL total reaction that included Power SYBR Green PCR mater mix (Applied Biosystems) and 0.625 μM forward and reverse primers (Table 2). Real-time PCR analysis was performed using a 7900HT fast real-time PCR system (Applied Biosystems). The PCR conditions consisted of an initial 2 min 50 °C step and a "hot start" step at 95 °C for 10 min, followed by 40 cycles of a 95 °C denaturing step for 15 s and a 60 °C annealing step for 60 s. A melting curve was analyzed for all primers to ensure the quality of the amplification product. Each sample was analyzed in duplicate for both the target and reference genes. Relative mRNA

**Table 2. Primers Used in the Q-PCR**

| gene | primer design | PCR product (bp) |
|---|---|---|
| ME | 5′-GGATAGGGCTGCTTTCAACA | 206 |
| | 3′-CTCCAGGGAACACGTAGGAA | |
| FASN | 5′- GCAGGGAAAATTCTGTGGAA | 200 |
| | 3′-CAGCGGTCAACAACAACATC | |
| Δ-9 desaturase | 5′-CCACCATACATTCCCCTACG | 176 |
| | 3′-CGCTCTTGTGACTCCCATCT | |
| Δ-6 desaturase | 5′- CGCATTCAGCAGATGAGTCT | 204 |
| | 3′-GCCGTAGGTGTCCTCATTGT | |
| β-actin | 5′-CACAATGTACCCTGGCATTG | 190 |
| | 3′-TCCGGATTCATCGTACTCCT | |



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**Table 3. Effects of Dietary Defatted Microalgae on the Plasma Fatty Acid Profile (as a Weight Percentage of Total Fatty Acids) at Week 6[a]**

| | DGA[b] (%) | | | | | | *p* value | | |
|---|---|---|---|---|---|---|---|---|---|
| fatty acid | 0 | 2 | 4 | 8 | 16 | SEM | ANOVA | linear[c] | $R^2$ |
| $C_{16:0}$ | 21.1 ab | 20.3 b | 19.9 b | 22.4 a | 21.0 ab | 0.29 | 0.06 | NS[d] | |
| $C_{16:1}$ | 0.73 c | 0.71 c | 1.15 bc | 1.49 a | 1.40 ab | 0.08 | 0.0004 | 0.005 | 0.30 |
| $C_{18:0}$ | 22.6 ab | 22.4 ab | 23.9 a | 21.5 b | 21.2 b | 0.29 | 0.01 | 0.08 | 0.12 |
| $C_{18:1n-9}$ | 13.4 ab | 12.2 ab | 14.1 ab | 14.0 a | 11.8 b | 0.30 | 0.09 | NS | |
| $C_{18:2n-6}$ | 33.6 ab | 34.7 a | 30.8 bc | 27.8 c | 31.0 abc | 0.69 | 0.006 | 0.10 | 0.11 |
| $C_{20:2n-6}$ | 0.31 | 0.28 | 0.25 | 0.28 | 0.17 | 0.03 | NS | NS | |
| $C_{20:3\ n-6}$ | 2.26 ab | 1.89 b | 2.65 a | 1.81 b | 1.18 c | 0.12 | 0.0004 | 0.002 | 0.34 |
| $C_{20:4n-6}$ | 4.89 | 5.27 | 4.99 | 5.57 | 5.34 | 0.18 | NS | NS | |
| $C_{20:5n-3}$ | 0.03 c | 0.44 c | 0.33 c | 1.69 b | 3.09 a | 0.22 | <0.0001 | <0.0001 | 0.87 |
| $C_{22:6n-3}$ | 0.37 c | 1.55 b | 1.77 b | 3.30 a | 3.48 a | 0.25 | <0.0001 | <0.0001 | 0.50 |
| total | | | | | | | | | |
| SFA | 44.1 | 42.7 | 43.9 | 44.0 | 42.2 | 0.35 | NS | NS | |
| MUFA | 14.7 ab | 13.0 b | 15.3 ab | 15.5 a | 13.3 b | 0.33 | 0.08 | NS | |
| PUFA | 41.7 ab | 44.3 ab | 40.8 ab | 40.6 b | 44.5 a | 0.59 | 0.08 | NS | |
| n-3 | 0.45 d | 2.10 c | 2.30 c | 5.04 b | 6.61 a | 0.45 | <0.0001 | <0.0001 | 0.75 |
| n-6 | 41.3 a | 42.2 a | 38.7 abc | 35.5 c | 37.9 bc | 0.68 | 0.005 | 0.02 | 0.22 |
| n-6/n-3 | 32.6 a | 20.1 b | 14.2 c | 6.83 d | 5.80 d | 1.71 | <0.0001 | <0.0001 | 0.53 |

[a]Data are expressed as the mean ($n$ = 6/treatment). Values with different letters in each row differ ($p < 0.05$). [b]DGA = defatted green microalgal biomass (*N. oceanica*, Cellana, Kailua-Kona, HI). [c]Data were analyzed using the linear regression model of SAS. [d]NS = not significant.

**Table 4. Effects of Dietary Defatted Microalgae on the Liver Fatty Acid Profile (as a Weight Percentage of Total Fatty Acids) at Week 6[a]**

| | DGA[b] (%) | | | | | | *p* value | | |
|---|---|---|---|---|---|---|---|---|---|
| fatty acid | 0 | 2 | 4 | 8 | 16 | SEM | ANOVA | linear[c] | $R^2$ |
| $C_{16:0}$ | 22.0 ab | 20.3 b | 20.8 b | 22.8 a | 20.9 b | 0.30 | 0.05 | 0.08 | 0.11 |
| $C_{16:1}$ | 1.35 | 1.15 | 1.36 | 1.74 | 1.44 | 0.07 | NS[d] | NS | |
| $C_{18:0}$ | 22.8 | 23.2 | 22.8 | 20.2 | 23.0 | 0.47 | NS | NS | |
| $C_{18:1n-9}$ | 21.3 | 17.2 | 19.9 | 22.7 | 18.5 | 0.72 | NS | NS | |
| $C_{18:2n-6}$ | 24.0 | 27.4 | 24.8 | 22.8 | 24.4 | 0.58 | NS | NS | |
| $C_{18:3n-6}$ | 0.46 | 0.59 | 0.53 | 0.47 | 0.40 | 0.04 | NS | 0.08 | 0.11 |
| $C_{18:3n-3}$ | 0.42 | 0.53 | 0.41 | 0.47 | 0.38 | 0.04 | NS | NS | |
| $C_{20:0}$ | 0.22 | 0.16 | 0.22 | 0.12 | 0.30 | 0.03 | NS | NS | |
| $C_{20:2n-6}$ | 0.40 | 0.43 | 0.43 | 0.30 | 0.40 | 0.02 | NS | NS | |
| $C_{20:3n-6}$ | 1.65 a | 1.44 ab | 1.67 a | 1.23 b | 1.31 b | 0.05 | 0.02 | 0.03 | 0.16 |
| $C_{20:4n-6}$ | 3.62 | 4.26 | 3.80 | 3.03 | 3.69 | 0.14 | NS | NS | |
| $C_{20:5\ n-3}$ | 0.06 c | 0.50 bc | 0.39 bc | 0.83 ab | 1.29 a | 0.10 | 0.0008 | <0.0001 | 0.47 |
| $C_{22:6\ n-3}$ | 1.04 b | 2.33 a | 2.14 a | 2.58 a | 3.23 a | 0.20 | 0.005 | 0.002 | 0.31 |
| total | | | | | | | | | |
| SFA | 45.3 | 43.9 | 44.2 | 43.6 | 44.6 | 0.51 | NS | NS | |
| MUFA | 22.9 | 18.6 | 21.6 | 24.7 | 20.3 | 0.78 | NS | NS | |
| PUFA | 31.7 | 37.5 | 34.2 | 31.7 | 35.1 | 0.83 | NS | NS | |
| n-3 | 1.57 c | 3.36 b | 2.94 bc | 3.89 ab | 4.90 a | 0.29 | 0.002 | 0.0004 | 0.38 |
| n-6 | 30.1 ab | 34.2 a | 31.2 ab | 27.8 b | 30.2 ab | 0.69 | 0.05 | 0.09 | 0.10 |
| n-6/n-3 | 21.6 a | 11.2 b | 11.4 b | 8.06 b | 7.20 b | 1.12 | <0.0001 | 0.0002 | 0.40 |

[a]Data are expressed as the mean ($n$ = 6/treatment). Values with different letters in each row differ ($p < 0.05$). [b]DGA = defatted green microalgal biomass (*N. oceanica*, Cellana, Kailua-Kona, HI). [c]Data were analyzed using the linear regression model of SAS. [d]NS = not significant.

abundance was determined using the Δcycle threshold ($\Delta C_t$) method. For each sample, the $C_t$ difference between the target and reference gene was calculated ($\Delta C_t = C_t^{\text{target}} - C_t^{\text{reference}}$). The $\Delta C_t$ values were then converted to fold differences by raising 2 to the power $-\Delta C_t$ ($2^{-\Delta C_t}$).

**Statistical Analyses.** Data were pooled within cage for an experimental unit of six per treatment and were analyzed by analysis of variation (ANOVA) and/or linear regression models using PC-SAS 9.2. Mean differences between dietary groups were separated using Duncan's multiple range test. For the gene expression data, selected treatment effects were directly compared to the control group using the *t* test. Data are expressed as the mean ± standard error of the mean (SEM), and the treatment effects were deemed significant at $p < 0.05$, with a trend at $p < 0.10$.

## RESULTS AND DISCUSSION

The animal performance and biochemical analyses, including plasma uric acid, inorganic phosphorus, and protein, for this experiment were reported elswhere.[35] Moderate levels (8%) of DGA were tolerated by chicks for 6 weeks without affecting growth performance or plasma biochemistry. However, the



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**Table 5. Effects of Dietary Defatted Microalgae on the Breast Fatty Acid Profile (as a Weight Percentage of Total Fatty Acids and mg/100 g of Sample) at Week 6[a]**

| | DGA[b] (%) | | | | | | *p* value | | |
|---|---|---|---|---|---|---|---|---|---|
| fatty acid | 0 | 2 | 4 | 8 | 16 | SEM | ANOVA | linear[c] | $R^2$ |
| $C_{14:0}$ | 0.59 b | 0.53 b | 0.50 b | 0.62 ab | 0.82 a | 0.02 | 0.03 | 0.02 | 0.19 |
| $C_{16:0}$ | 23.0 | 22.6 | 23.4 | 23.4 | 22.4 | 0.17 | NS[d] | NS | |
| $C_{16:1}$ | 3.13 cd | 2.99 d | 3.36 bc | 4.22 ab | 4.68 a | 0.16 | <0.0001 | <0.0001 | 0.55 |
| $C_{18:0}$ | 8.73 | 8.36 | 8.65 | 8.11 | 7.82 | 0.20 | NS | NS | |
| $C_{18:1n-9}$ | 30.9 | 31.4 | 30.8 | 31.3 | 29.9 | 0.40 | NS | NS | |
| $C_{18:2n-6}$ | 29.1 | 29.7 | 28.9 | 27.6 | 29.1 | 0.29 | NS | NS | |
| $C_{18:3n-6}$ | 0.25 | 0.27 | 0.24 | 0.24 | 0.20 | 0.03 | NS | NS | |
| $C_{18:3n-3}$ | 0.94 a | 1.00 a | 0.68 b | 0.89 ab | 0.85 ab | 0.03 | 0.04 | NS | |
| $C_{20:2n-6}$ | 0.42 | 0.39 | 0.36 | 0.53 | 0.36 | 0.06 | NS | NS | |
| $C_{20:3n-6}$ | 1.03 | 0.86 | 0.80 | 0.76 | 0.72 | 0.04 | NS | 0.10 | 0.11 |
| $C_{20:4n-6}$ | 1.44 | 1.21 | 1.27 | 1.19 | 1.31 | 0.16 | NS | NS | |
| $C_{20:5n-3}$ | 0.00 d | 0.06 cd | 0.08 c | 0.39 b | 0.54 a | 0.06 | <0.0001 | <0.0001 | 0.86 |
| $C_{22:6n-3}$ | 0.01 c | 0.18 bc | 0.34 b | 0.62 a | 0.79 a | 0.06 | <0.0001 | <0.0001 | 0.78 |
| total (%) | | | | | | | | | |
| SFA | 32.5 | 31.6 | 32.6 | 32.2 | 31.1 | 0.32 | NS | NS | |
| MUFA | 34.4 | 34.7 | 34.7 | 35.8 | 34.9 | 0.50 | NS | NS | |
| PUFA | 33.2 | 33.7 | 32.7 | 32.0 | 34.0 | 0.37 | NS | NS | |
| n-3 | 0.96 d | 1.23 c | 1.24 c | 1.89 b | 2.18 a | 0.13 | <0.0001 | <0.0001 | 0.76 |
| n-6 | 32.2 | 32.4 | 31.6 | 30.1 | 31.8 | 0.37 | NS | NS | |
| mg/100 g | | | | | | | | | |
| total | 1319 | 1256 | 1329 | 1222 | 1343 | 37.1 | NS | NS | |
| SFA | 418 | 394 | 429 | 390 | 413 | 10.4 | NS | NS | |
| MUFA | 461 | 439 | 466 | 442 | 479 | 15.5 | NS | NS | |
| PUFA | 439 | 423 | 434 | 390 | 451 | 12.2 | NS | NS | |

[a]Data are expressed as the mean ($n$ = 6/treatment). Values with different letters in each row differ ($p < 0.05$). [b]DGA = defatted green microalgal biomass (*N. oceanica*, Cellana, Kailua-Kona, HI). [c]Data were analyzed using the linear regression model of SAS. [d]NS = not significant.

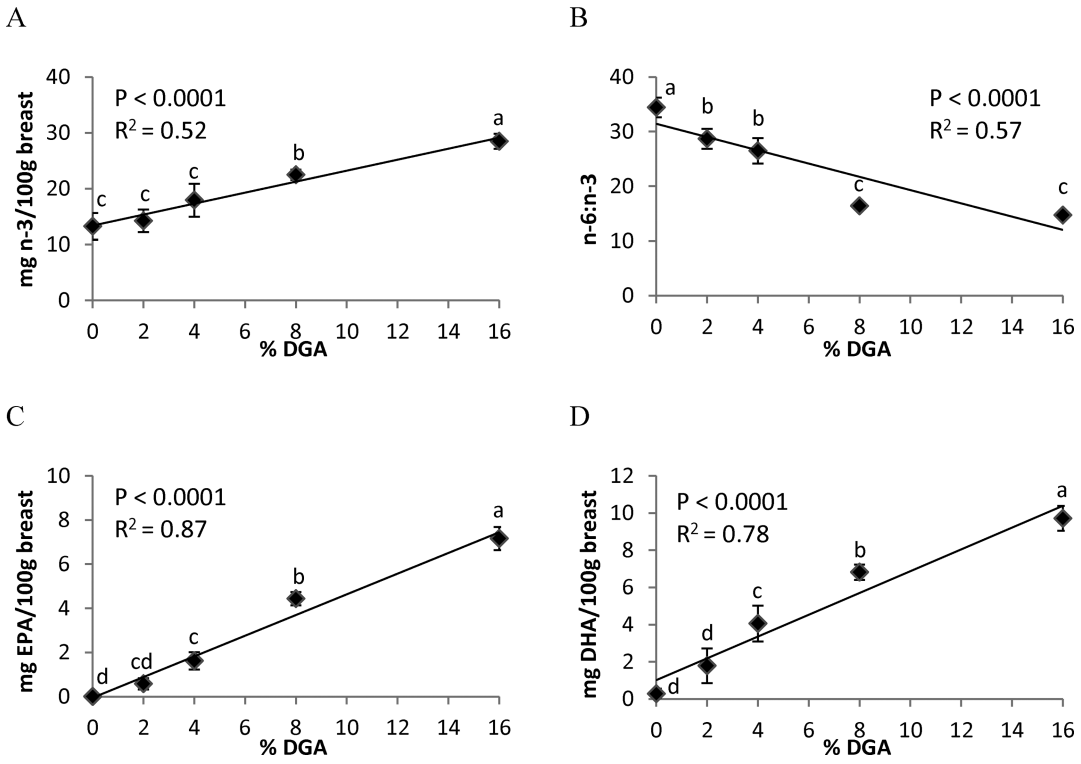


**Figure 1.** Effects of dietary inclusion of defatted microalgal biomass on the n-3 fatty acid profile of broiler chicken breast at week 6: (a) n-3 fatty acids, (b) n-3/n-6 fatty acid ratio, (c) EPA, and (d) DHA. Data are expressed as the mean ± SEM ($n$ = 6/treatment). Values with different letters in each group differ significantly according to one-way ANOVA ($p < 0.05$). Linear regression analysis was also deemed significant at $p < 0.05$. DGA = defatted green microalgal biomass (*N. oceanica*, Cellana, Kailua-Kona, HI).



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**Table 6. Effects of Dietary Defatted Microalgae on the Thigh Fatty Acid Profile (as a Weight Percentage of Total Fatty Acids and mg/100 g of Sample) at Week 6[a]**

| | DGA[b] (%) | | | | | | *p* value | | |
|---|---|---|---|---|---|---|---|---|---|
| fatty acid | 0 | 2 | 4 | 8 | 16 | SEM | ANOVA | linear[c] | $R^2$ |
| $C_{14:0}$ | 0.39 b | 0.43 b | 0.52 a | 0.56 a | 0.59 a | 0.02 | 0.0002 | <0.0001 | 0.45 |
| $C_{16:0}$ | 22.9 | 23.3 | 22.4 | 22.6 | 22.0 | 0.17 | NS[d] | 0.03 | 0.16 |
| $C_{16:1}$ | 2.33 c | 2.43 c | 3.19 b | 3.80 ab | 4.08 a | 0.16 | <0.0001 | <0.0001 | 0.58 |
| $C_{18:0}$ | 11.0 a | 10.7 ab | 10.2 ab | 9.55 b | 9.66 b | 0.20 | 0.06 | 0.01 | 0.21 |
| $C_{18:1n-9}$ | 26.5 | 25.4 | 27.3 | 27.3 | 26.3 | 0.40 | NS | NS | |
| $C_{18:2n-6}$ | 27.8 | 28.6 | 27.5 | 27.4 | 27.0 | 0.29 | NS | NS | |
| $C_{18:3n-6}$ | 0.23 | 0.20 | 0.31 | 0.32 | 0.39 | 0.03 | NS | 0.07 | 0.12 |
| $C_{18:3n-3}$ | 0.35 b | 0.57 a | 0.57 a | 0.56 a | 0.53 ab | 0.03 | 0.04 | NS | |
| $C_{20:1n-9}$ | 0.77 a | 0.62 ab | 0.50 ab | 0.46 b | 0.33 b | 0.05 | 0.03 | 0.003 | 0.28 |
| $C_{20:2n-6}$ | 0.68 | 0.52 | 0.60 | 0.52 | 0.56 | 0.06 | NS | NS | |
| $C_{20:3n-6}$ | 1.19 a | 1.12 ab | 0.93 bc | 0.88 c | 0.83 c | 0.04 | 0.008 | 0.001 | 0.31 |
| $C_{20:4n-6}$ | 5.03 | 5.00 | 4.57 | 4.31 | 4.93 | 0.16 | NS | NS | |
| $C_{20:5n-3}$ | 0.00 d | 0.12 cd | 0.23 c | 0.44 b | 0.88 a | 0.06 | <0.0001 | <0.0001 | 0.84 |
| $C_{22:6n-3}$ | 0.12 d | 0.42 c | 0.56 c | 0.76 b | 1.04 a | 0.06 | <0.0001 | <0.0001 | 0.74 |
| total (%) | | | | | | | | | |
| SFA | 34.9 | 34.9 | 33.7 | 33.2 | 32.8 | 0.32 | NS | 0.01 | 0.20 |
| MUFA | 29.7 | 28.5 | 31.1 | 31.6 | 31.0 | 0.50 | NS | NS | |
| PUFA | 35.4 | 36.6 | 35.2 | 35.2 | 36.2 | 0.37 | NS | NS | |
| n-3 | 0.46 d | 1.11 c | 1.36 c | 1.76 b | 2.45 a | 0.13 | <0.0001 | <0.0001 | 0.80 |
| n-6 | 35.0 | 35.5 | 33.9 | 33.4 | 33.7 | 0.37 | NS | NS | |
| mg/100 g | | | | | | | | | |
| total | 758 | 795 | 976 | 986 | 956 | 37.1 | NS | 0.08 | 0.11 |
| SFA | 261 | 270 | 323 | 324 | 313 | 10.4 | NS | 0.10 | 0.10 |
| MUFA | 228 | 237 | 308 | 323 | 300 | 15.5 | NS | NS | |
| PUFA | 270 | 290 | 346 | 343 | 346 | 12.2 | NS | 0.05 | 0.13 |

[a]Data are expressed as the mean ($n$ = 6/treatment). Values with different letters in each row differ ($p < 0.05$). [b]DGA = defatted green microalgal biomass (*N. oceanica*, Cellana, Kailua-Kona, HI). [c]Data were analyzed using the linear regression model of SAS. [d]NS = not significant.

higher level of inclusion (16%) led to a reduction in indices of growth performance.

The fatty acid composition of the defatted microalgal biomass and experimental diet is shown in Table 1. Containing 3.6% total lipid, the DGA was rich in EPA (representing 16.5% of total fatty acids by weight) but without DHA. The fatty acid profile (as a weight percentage of total fatty acids) of week 6 plasma is shown in Table 3 (week 3 is shown on Table S4). The main fatty acid found in all dietary treatments at both time points was linoleic acid (C18:2n-6), followed by palmitic acid (C16:0) and stearic acid (C18:0), which are similar. There was no effect of DGA inclusion on saturated fatty acids (SFAs), monounsaturated fatty acids (MUFAs), or PUFAs, regardless of time. In contrast, the DGA inclusion resulted in a linear increase in plasma n-3 fatty acids, and the increases were 5- and 15-fold by the 16% DGA diet over the control at weeks 3 ($p < 0.0001$; $R^2 = 0.93$) and 6 ($p < 0.0001$; $R^2 = 0.75$), respectively. The increases were manifested with elevated EPA (C20:5n-3) and DHA (C22:6n-3), indicating that the birds were able to convert the dietary EPA into DHA. At week 6, there was a linear reduction in n-6 fatty acids ($p < 0.05$), resulting in a favorable decrease in the ratio of n-6/n-3 fatty acids ($p < 0.0001$).

The DGA inclusion exerted a strong effect (from $p < 0.05$ to $p < 0.0001$) on the liver fatty acid profiles (as weight percentage of total fatty acids) of week 6 (Table 4) and week 3 (Table S5). Although the four main fatty acids in liver, palmitic acid, stearic acid, oleic acid (C18:1n-9), and linoleic acid, and the percentages of total SFAs, MUFAs, and PUFAs were not affected by DGA inclusion at week 6, total liver n-3 fatty acids displayed a linear increase ($p < 0.001$; $R^2 = 0.38$) to the inclusion doses. The actual increase was 3.1-fold by the 16% DGA diet compared to the control. Similar to the plasma, the increases in n-3 fatty acids were attributed to the elevations of both EPA ($p < 0.0001$; $R^2 = 0.47$) and DHA ($p = 0.002$; $R^2 = 0.31$). There was a trend ($p = 0.09$) of a linear decrease in n-6 fatty acids, leading to a corresponding linear decrease in the n-6/n-3 fatty acid ratio ($p = 0.0002$; $R^2 = 0.40$). Similar results were shown at week 3 (Table S5).

Table 5 shows the breast muscle fatty acid profile (as a weight percentage of total fatty acids and mg/g of wet tissue) of week 6. The predominant fatty acids in this tissue were not affected by the DGA inclusion either and were oleic and linoleic acids, followed by palmitic acid. Similar to the results shown in the plasma and liver, the DGA inclusion did not alter total SFAs, MUFAs, or PUFAs but caused a linear increase in n-3 fatty acids ($p < 0.0001$; $R^2 = 0.76$) in the breast muscle. Similar results were shown for week 3 (Table S6). Dietary DGA inclusion had no effect on total fat, SFAs, MUFAs, or PUFAs (expressed as milligrams per 100 g of wet muscle sample) but linearly increased n-3 fatty acids ($p < 0.0001$; $R^2 = 0.52$), EPA ($p < 0.0001$; $R^2 = 0.87$), and DHA ($p < 0.0001$; $R^2 = 0.78$) and decreased the n-6/n-3 fatty acid ratio ($p < 0.0001$; $R^2 = 0.57$) (Figure 1). Total combined EPA and DHA in 100 g of breast muscle tissue reached 16.9 mg in chicks fed the 16% DGA diet, which represented an over 60-fold increase compared to that of the chicks fed the control diet. Consistent with our findings, broiler chicks fed full-fatted golden marine algae[16] and DHA-rich microalgae[18,36] displayed increased total n-3 fatty acids and



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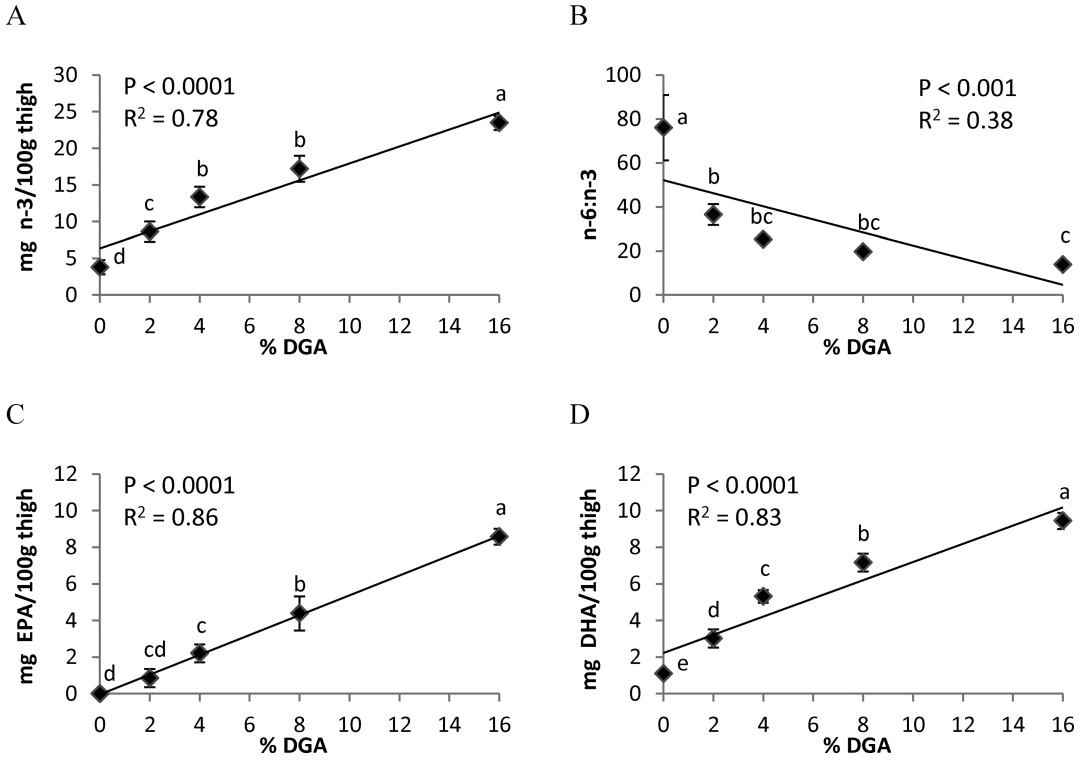


**Figure 2.** Effects of dietary inclusion of defatted microalgal biomass on the n-3 fatty acid profile of broiler chicken thigh at week 6: (a) n-3 fatty acids, (b) n-3/n-6 fatty acids, (c) EPA, and (d) DHA. Data are expressed as the mean ± SEM ($n$ = 6/treatment). Values with different letters in each group differ significantly according to one-way ANOVA ($p < 0.05$). Linear regression analysis was also deemed significant at $p < 0.05$. DGA = defatted green microalgal biomass (*N. oceanica*, Cellana, Kailua-Kona, HI).

decreased n-6/n-3 fatty acid ratio in the breast muscle compared to those consuming a control ratio.

The predominant fatty acids (as a weight percentage of total fatty acids and mg/g of muscle) in the thigh were similar to those found in the breast tissue (Table 6). Interestingly, there was a trend ($p$ = 0.09) at week 3 and a linear reduction ($p < 0.01$; $R^2$ = 0.20) at week 6 in percentage of SFA, respectively, over the increasing doses of the DGA inclusion. However, there was no effect of DGA inclusion on the percentage of thigh MUFAs or PUFAs, whereas the inclusion at week 6 led to a linear increase in percentage of n-3 fatty acids ($p < 0.0001$; $R^2$ = 0.80). Similar results were shown at week 3 (Table S7). When expressed as mg/100 g of tissue, there was an increase in thigh muscle PUFAs ($p$ = 0.05) and a trend for an increase in total fat ($p$ = 0.08) and SFAs ($p$ = 0.10) with increasing DGA inclusion at week 6. The change in SFAs was presumably the result of an increase in dietary SFAs. Increasing the DGA inclusion also decreased linearly the ratio of n-6/n-3 fatty acids ($p < 0.001$; $R^2$ = 0.38), resulting in a 5.5-fold decline by the 16% DGA diet over the control diet (Figure 2). Meanwhile, there was a linear increase in n-3 fatty acids ($p < 0.0001$; $R^2$ = 0.78), EPA ($p < 0.0001$; $R^2$ = 0.86), and DHA ($p < 0.0001$; $R^2$ = 0.83) (Figure 2). Total combined EPA and DHA in 100 g of thigh muscle from the chicks fed the 16% DGA diet reached 18 mg, which was a 16.5-fold increase from the control.

Increasing the DGA inclusion elevated total dietary n-3 fatty acids by its contribution of EPA, while being completely devoid of DHA. However, the DGA inclusion elevated tissue deposition of both EPA and DHA. Interestingly, the deposition of DHA was numerically (1.12−2.50-fold) higher than that of EPA in tissues of chicks fed the 16% DGA diet, indicating an efficient *in vivo* conversion of EPA to DHA and/or upregulated *de novo* synthesis of DHA. Similarly, other researchers have reported higher concentrations of DHA, relative to EPA, in chicken muscle tissue[37] and egg yolk,[37,38] when the animals were fed supplemental fish oil that contained higher EPA than DHA. These findings, along with ours, reinforce the view that growing chickens are able to deposit n-3 fatty acids in tissues, specifically converting EPA into DHA, probably as a result of their greater efficiency of fatty acid elongases compared to other animal species.[39]

Genes involved in hepatic lipogenesis, such as FASN and ME, are known to be nutritionally controlled,[26,27,40] and altered regulation of these genes is associated with several diseases.[40] While the DGA inclusion did not affect the hepatic gene expression of ME (Table 7), there was an increase ($p < 0.10$) in mRNA of FASN in chicks consuming the 8% DGA diet compared to the control. While only evident in this one dietary

**Table 7. Effects of Dietary Defatted Microalgae on Liver Gene Expression at Week 6[a]**

| | DGA[b] (%) | | | | | |
|---|---|---|---|---|---|---|
| gene | 0 | 2 | 4 | 8 | 16 | SEM |
| ME[c] | 1.00 | 1.25 | 1.76 | 1.19 | 1.43 | 0.17 |
| FASN[c] | 1.00 | 1.15 | 1.22 | 1.35[d] | 1.04 | 0.09 |
| Δ-9 desaturase | 1.00 | 1.39 | 1.49 | 1.88[e] | 1.34 | 0.13 |
| Δ-6 desaturase | 1.00 | 1.42 | 1.96[e] | 1.75[d] | 1.35 | 0.15 |

[a]Data are expressed as the mean ($n$ = 6/treatment) and normalized to the control. [b]DGA = defatted green microalgal biomass (*N. oceanica*, Cellana, Kailua-Kona, HI). [c]ME, malic enzyme; FASN, fatty acid synthase. [d]$p < 0.10$ compared to the control. [e]$p < 0.05$ compared to the control.



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treatment, these data are in disagreement with others who have found that increasing consumption of PUFAs leads to a subsequent decrease in FASN expression.[27,41] However, those experiments used diets containing 20% menhaden oil. It is possible that our diets did not contain high enough levels of PUFAs to elicit a similar response. The cause for the increase in the FASN gene expression with only the consumption of the 8% DGA diet needs to be investigated further.

Desaturase enzymes, which introduce double bonds into long-chain fatty acids, produce unsaturated fatty acids that are essential for cellular functions. It is well-known that PUFAs are a main dietary regulator of these enzymes.[30,42] Specifically, expression of Δ-9 and Δ-6 desaturases, the enzymes responsible for the catalysis of the synthesis of MUFAs and PUFAs, respectively, is typically blunted in the presence of PUFA supplementation.[42−44] In the present experiment, there was no overall linear or quadratic regression in the gene expression with the increasing DGA inclusion. However, chicks fed the 8% DGA diet displayed elevated Δ-9 desaturase expression ($p < 0.05$) compared to those fed the control diet. Chicks fed both the 4% ($p < 0.05$) and 8% ($p < 0.10$) DGA diets also showed an increased Δ-6 desaturase gene expression over those fed the control diet. There was also a correlation between the chick body weight and liver Δ-9 desaturase mRNA level at week 6 (data not shown; $p = 0.09$; $R^2 = 0.34$). The gene expression of the lipogenic FASN was positively correlated with both Δ-9 ($p = 0.10$; $R^2 = 0.35$) and Δ-6 desaturase ($p = 0.007$; $R^2 = 0.54$) mRNA levels (data not shown). Nevertheless, the responses of these four genes involved in lipid and fatty acid metabolism to the dietary DGA inclusion were not correlated with those of the n-3 fatty acid enrichment or the ratios of n-6/n-3 fatty acids in plasma or tissues of chicks. Our ongoing research is exploring the underlying mechanism for the lack of these correlations.

In summary, our study indicates that the defatted microalgal biomass *N. oceanica* could be supplemented in the corn−soybean meal diet for broiler chicks to enhance EPA, DHA, and total n-3 fatty acids and decreased the n-6/n-3 fatty acid ratio in all tissues measured. While the highest level of DGA produced the most dramatic results, as little as 2% inclusion of the biomass was sufficient to produce detectable positive impacts on these measures. This highlights the feasibility in creating a novel chicken product enriched with n-3 fatty acids, in particular DHA and EPA, by feeding chicks the defatted microalgal biomass from the biofuel production. Ultimately, future research is warranted to illustrate the regulation of key genes and pathways involved in the biosynthesis of EPA and DHA and lipogenesis by the DGA inclusion to completely understand and apply this unique potential of the biomass in producing healthy, value-added animal foods.

## ■ ASSOCIATED CONTENT

### Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.jafc.5b03137.

Nutrient composition of the defatted green microalgal biomass (Table S1), formula and nutrient composition of starter diets (Table S2), formula and nutrient composition of grower diets (Table S3), effects dietary defatted microalgae on plasma fatty acid profile (as a weight percentage of total fatty acids) at week 3 (Table S4), effects dietary defatted microalgae on liver fatty acid profile (as a weight percentage of total fatty acids) at week 3 (Table S5), effects of dietary defatted microalgae on breast fatty acid profile (as a weight percentage of total fatty acids and mg/100 g of sample) at week 3 (Table S6), and effects of dietary defatted microalgae on thigh fatty acid profile (as a weight percentage of total fatty acids and mg/100 g of sample) at week 3 (Table S7) (PDF)

## ■ AUTHOR INFORMATION

### Corresponding Author

*Telephone: 607-254-4703. Fax: 607-255-9829. E-mail: xl20@cornell.edu.

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### Notes

The authors declare no competing financial interest.

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