**Functional Characterization of the Duck and Turkey Fatty Acyl Elongase Enzymes ELOVL5 and ELOVL2**

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**Abstract**

In most Western countries, the consumption of fish is low and insufficient to provide the recommended daily intake of the n–3 (ω-3) long-chain polyunsaturated fatty acids (PUFAs) eicosapentaenoic acid (EPA; 20:5n–3) and docosahexaenoic acid (DHA; 22:6n–3). Poultry has the potential to be a sustainable source of EPA and DHA if poultry species are capable of synthesizing these n–3 PUFAs from dietary plant-derived α-linolenic acid (ALA; 18:3n–3). In most animals, the elongation of very long-chain fatty acids (ELOVL) enzyme ELOVL2 is essential for conversion of dietary ALA to DHA because only ELOVL2 and not ELOVL5 can elongate docosapentaenoic acid (DPA; 22:5n–3) to 24:5n–3, the precursor of DHA. The chicken is the only poultry species in which elongase enzymes have been functionally characterized, and chicken ELOVL5 had unique DPA-to-24:5n–3 activity, which may enable chickens to synthesize more DHA than other animals. By using a yeast expression system, we examined the duck and turkey elongases, ELOVL2 and ELOVL5, to understand if all poultry species have similar potential to synthesize EPA and DHA. The duck and turkey ELOVL5 enzymes were active with C18–20 PUFAs only. The duck ELOVL2 had a broad substrate specificity with C18–22 PUFAs, whereas the turkey ELOVL2 was active only with EPA and C22 PUFAs. Both duck and turkey ELOVL2 enzymes catalyzed 2 rounds of EPA elongation, with the products being DPA and its elongation product, 24:5n–3. With exogenous DPA, both duck and turkey ELOVL2 synthesized 24:5n–3, with the duck ELOVL2 being more active than the turkey ELOVL2. The reason for the lack of DPA elongation activity by the duck and turkey ELOVL5 enzymes compared with the chicken ELOVL5 could not be elucidated by protein sequence comparisons. By using the elongase enzyme activities only as a predictor of DHA synthesis, ducks may have a similar ability to chickens to convert increasing dietary ALA to DHA. J. Nutr. 144: 1234–1239, 2014.

**Introduction**

Ingestion of fish or fish oil is the most direct method of elevating tissue concentrations of the n–3 long-chain PUFAs (LCPUFAs)3 EPA and DHA. The beneficial health effects of EPA and DHA have driven human use to an extent that there are concerns that fish and fish oil cannot meet this demand. Poultry could be an alternate and sustainable source of EPA and DHA, provided that these FAs could be synthesized from dietary plant-derived α-linolenic acid (ALA; 18:3n–3). Metabolism of ALA to EPA and DHA requires progressive desaturation and elongation. Our previous findings in rats indicate that the elongation of the very long-chain FAs (ELOVL) enzyme ELOVL2 is essential for the conversion of dietary ALA to DHA because only ELOVL2 and not ELOVL5 can elongate the 22-carbon substrate docosapentaenoic acid (DPA; 22:5n–3) to 24:5n–3, which is necessary for DHA synthesis (1). A recent study using Elovl2−/− mice reaffirmed the essentiality of ELOVL2 for DHA synthesis in rodents (2). We reported that chicken ELOVL5 was unlike rodent ELOVL5 in that it could elongate DPA to 24:5n–3, which together with the ELOVL2 DPA elongation activity may enable chickens to synthesize more 24:5n–3 and subsequently more DHA than other species (3).

Chickens and turkeys were the most common global source of poultry meat in 2007 with 87% and 6.7% of total poultry production, respectively (4). Duck meat production followed at 4% of total poultry production worldwide, but it is particularly important in the diets of China and Southeast Asian countries (4). The chicken is the only poultry species in which fatty acyl elongase enzymes have been functionally characterized. This study examined the activities of the duck and turkey elongases, ELOVL2 and ELOVL5, by using a yeast heterologous expression system. The duck ELOVL2 had a broad substrate specificity with C18–22 PUFAs, whereas the turkey ELOVL2 was active only with EPA and C22 PUFAs. Both duck and turkey ELOVL2 enzymes catalyzed 2 rounds of EPA elongation, with the products being DPA and its elongation product, 24:5n–3. With exogenous DPA, both duck and turkey ELOVL2 synthesized 24:5n–3, with the duck ELOVL2 being more active than the turkey ELOVL2. The reason for the lack of DPA elongation activity by the duck and turkey ELOVL5 enzymes compared with the chicken ELOVL5 could not be elucidated by protein sequence comparisons. By using the elongase enzyme activities only as a predictor of DHA synthesis, ducks may have a similar ability to chickens to convert increasing dietary ALA to DHA. J. Nutr. 144: 1234–1239, 2014.

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2 Supplemental Table 1 is available from the “Online Supporting Material” link in the online posting of the article and from the same link in the online table of contents at http://jn.nutrition.org.

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system to better understand if these commercial birds could be a useful population source of EPA and DHA.

**Materials and Methods**

This study was approved by the Animal Ethics Committees of SA Pathology/Central Adelaide Local Health Network, South Australia, and the University of Adelaide, South Australia.

**Cloning the duck and turkey ELOVL2 and ELOVL5 cDNA.** Total RNA was extracted from the liver of domestic Pekin ducks (*Anas platyrhynchos domesticus*) or Australian white hybrid turkeys (*Meleagris gallopavo*) with the RNeasy kit (Qiagen). RT-PCR amplification of the ELOVL2 and ELOVL5 open reading frames was performed by using the primers listed in Supplemental Table 1 and cloned into the expression vector pYES2 (Invitrogen) as previously described (1).

**Heterologous expression of the duck and turkey ELOVL2 and ELOVL5 in Saccharomyces cerevisiae.** The pYES2-ELOVL2 and pYES2-ELOVL5 constructs were used to transform the *Saccharomyces cerevisiae* strain INVSc1 for the production of recombinant protein. Transformation, selection, and maintenance of *S. cerevisiae* were performed as previously described (1). PUFAs were obtained from Cayman Chemical Company (Sapphire Bioscience) and were diluted in analytical-grade ethanol (Sigma) to prepare 500 μmol/L stock solutions, which were stored at −20°C. Recombinant yeast expressing ELOVL2 or ELOVL5 were supplemented with 100 μmol/L of the following PUFAs substrates: 18:4n–3 (stearidonic acid), 18:5n–6 (γ-linolenic acid), 20:5n–3 (EPA), 20:4n–6 (arachidonic acid), 22:5n–3 (DPA), or 22:4n–6. Dose-response curves were generated by supplementing recombinant yeast expressing ELOVL2 or ELOVL5 with 100–400 μmol/L of EPA or DPA. The volume of ethanol in each incubation was kept constant. Cells were harvested for analysis after 24 h of FA supplementation at 30°C. Data are the means ± SDs of triplicate incubations.

**FA analysis.** Total lipid was extracted from yeast cells by using chloroform: methanol (2:1, v:v) and methylated in 1% sulfuric acid in methanol (v:v) at 70°C for 3 h to prepare FAME. FAME were then extracted and analyzed by GC as previously described (5). The identity of each FA peak in the chromatogram was ascertained by comparing its retention time to authentic lipid standards (NuChek Prep; Larodan Fine Chemicals). The identities of 20:4n–3 and 22:4n–3 were confirmed by GC-MS (5). The amount of each FA was expressed either in nmol FA/g yeast or as a proportion of FA substrate. The proportion of FA substrate converted to FA product(s) (% conversion) and calculated as [product(s)/ (product(s) + substrate)] × 100. The internal FFA standard 17:0 was used to quantify the amount of each endogenous FA. The limit of detection was 0.05% of total FAs.

**Statistical analysis.** FA data obtained from the expression of ELOVL2 or ELOVL5 in *S. cerevisiae* were analyzed by 1-factor ANOVA with Tukey’s post hoc test. Unpaired t test was used to identify differences between duck and turkey corresponding values. The 1-factor ANOVA and t test analyses assumed Gaussian distribution, although distribution was not tested because of sample size (n = 3). Analyses were carried out using Graphpad Prism version 5.03 for Windows (Graphpad Software). Statistical significance was set at P < 0.05 in all tests.

**Results**

**Sequence analysis of the duck ELOVL2 and ELOVL5.** An 891 bp ELOVL2 open reading frame was amplified and aligned with the predicted duck *ELOVL2* sequence in GenBank (XM_005016953). The amplified sequence was 186 nucleotides shorter at the 5′ end than sequence XM_005016953, and there were 10 nucleotide differences between the aligned 891 bp. The duck *ELOVL2* sequence was deposited in GenBank as accession number KJ409554. Amplification of the 888 bp *ELOVL5* open reading frame and subsequent alignment with the predicted duck *ELOVL5* sequences in GenBank (XM_005009189 and XM_005009190) revealed 1 nucleotide difference. The duck *ELOVL5* sequence was deposited in GenBank as accession number KJ409553. The predicted ELOVL5 protein of 295 amino acids was identical to XP_005009246 and XP_005009247.

**FIGURE 1** Duck (A) and turkey (B) ELOVL5 and duck (C) and turkey (D) ELOVL2 substrate specificities after expression in *Saccharomyces cerevisiae* and supplemented with 100 μmol/L of various C18–22 PUFAs. The proportion of FA substrate converted to FA product(s) is the percent conversion. Values are means ± SDs, n = 3. Labeled means without a common letter differ, P < 0.05. *Conversion includes the 4-carbon elongation product. The limit of detection was 0.05% of total FAs. AA, arachidonic acid (20:4n–6); EPA, docosapentaenoic acid (22:5n–3); DPA, elongation of very long-chain FAs; GLA, γ-linolenic acid (18:3n–6); ND, not detected; SDA, stearidonic acid (18:4n–3).
Sequence analysis of the turkey ELOVL2 and ELOVL5. An 894 bp ELOVL2 open reading frame was amplified and aligned with the predicted turkey ELOVL2 sequence in Genbank (XM_003204749). The amplified sequence was 48 nucleotides shorter at the 5' end than sequence XM_003204749, and there were 2 nucleotide differences between the aligned 894 bp. The turkey ELOVL2 sequence was deposited in GenBank as accession number KJ409552. The predicted ELOVL5 protein of 295 amino acids was identical to XP_003204479.

Comparison of duck and turkey ELOVL2 and ELOVL5 substrate specificities. Recombinant S. cerevisiae cells expressing ELOVL2 or ELOVL5 were cultured in the presence of 100 μmol/L of various n-3 and n-6 C18–22 PUFAs to determine substrate specificities of the elongase enzymes. The duck and turkey ELOVL5 enzymes were active with C18 and C20 PUFAs, but not C22 PUFAs (Fig. 1A, B). The duck ELOVL2 had a broad substrate specificity with C18, C20, and C22 PUFAs, with the least activity being with the C18 PUFAs (Fig. 1C). In contrast, the turkey ELOVL2 was active only with the C20 PUFA EPA and the C22 PUFAs (Fig. 1D). Both duck and turkey ELOVL2 enzymes had a clear preference for n-3 over n-6 PUFA substrates.

Examination of the products of EPA and DPA elongation by ELOVL2 and ELOVL5. The products of EPA elongation were examined by using EPA and DPA dose-response curves. For both duck and turkey ELOVL5 there was a proportional increase in DPA synthesis with increasing concentrations of EPA, but 24:5n-3 synthesized from endogenous DPA was negligible (Fig. 2A, B). Likewise, both duck and turkey ELOVL2 elongated EPA to DPA in a dose-dependent manner, but in contrast to the ELOVL5 activity, duck and turkey ELOVL2 elongated the endogenously formed DPA to 24:5n-3. In fact, 24:5n-3 was the major product of EPA elongation by both duck and turkey ELOVL2 enzymes (Fig. 2C, D). Duck ELOVL2 was more active than turkey ELOVL2 in elongating endogenously formed DPA, with a dose-dependent increase in 24:5n-3 up to 200 μmol/L of DPA and then apparent saturation at higher EPA concentrations (Fig. 2C).

When DPA was used as an exogenous substrate, the duck ELOVL2 synthesized the same amount of 24:5n-3 regardless of the
DPA concentration above 100 μmol/L (Fig. 3A). Saturation of conversion of DPA to 24:5n–3 was also observed with turkey ELOVL2 although at lower concentrations than for duck ELOVL2 (Fig. 3B).

**Comparison of poultry ELOVL2 and ELOVL5 predicted proteins.** Comparisons of the deduced amino acid sequences of duck, turkey, and chicken ELOVL5 revealed that turkey and chicken ELOVL5 were the most similar with 99% identity, followed by chicken and duck ELOVL5 with 98% identity, and turkey and duck ELOVL5 with 97% identity (Fig. 4). A tryptophan at position 231 is a characteristic feature of all ELOVL5 proteins and was conserved in the ELOVL5 predicted protein sequences from each of the birds (Fig. 4).

Comparisons of the deduced amino acid sequences of duck, turkey, and chicken ELOVL2 revealed that turkey and chicken ELOVL2 were the most similar with 97% identity, followed by turkey and duck with 94% identity, and the least similar were chicken and duck with 93% identity (Fig. 5). A cysteine at position 234 is a characteristic feature of all ELOVL2 proteins and was conserved in the ELOVL2 predicted protein sequences from each of the birds (Fig. 5).

**Discussion**

The main sources of dietary n–3 FAs are vegetable oils, which contain ALA, and fish and fish oil, which contain EPA and DHA. In most Western countries, the consumption of fish is low (6). Authoritative bodies across the United States (7), Europe (8), and Australia (9) have issued guidelines or advice on EPA and DHA intake. These recommended daily intakes are varied and encourage a healthy population to consume at least 200 mg of EPA and DHA in Europe (8) or 500 mg of EPA and DHA in Australia (9). In populations with coronary heart disease, the recommended daily intake is 1000 mg of EPA and DHA (7,9).

Worldwide the production of poultry continues to increase at a higher rate than other land production animals such as pig, ovine, and bovine (10). In 2013, poultry production increased by 1.8% from the previous year, whereas pig, ovine, and bovine production increased 1.7%, 1.5%, and 0.2%, respectively (10). The acceptance of poultry in the human diet lends itself to be a useful population source of EPA and DHA if poultry species are capable of synthesizing these n–3 LCPUFAs from dietary ALA. Chickens are the only poultry species in which the elongase enzymes involved in LCPUFA synthesis have been functionally characterized (3). In this study, we investigated the duck and turkey elongase enzymes to understand if all poultry species possess the same potential to synthesize n–3 LCPUFAs.

The duck and turkey ELOVL5 activities were limited to C18 and C20 PUFA substrates, as found in most vertebrate (1,5,11–20) and invertebrate ELOVL5 enzymes (21). The duck and turkey ELOVL5 enzyme activities were different from the chicken ELOVL5, which has unique DPA to 24:5n–3 activity (3). The

**FIGURE 4** A deduced amino acid sequence alignment of turkey, chicken, and duck ELOVL5. Identity/similarity shading was based on the Gonnet series matrix produced by ClustalX where primary black shading indicates identical residues and secondary and tertiary gray shading indicates similar residues with an 80% and 60% cut off, respectively. Seven predicted transmembrane-spanning domains were predicted by using the HMMTOP transmembrane topology prediction server (version 2.0) and are shown with a solid-lined box. The conserved tryptophan in all ELOVL5 sequences is indicated with an arrow. ELOVL, elongation of very long-chain FAs.

**FIGURE 5** A deduced amino acid sequence alignment of turkey, chicken, and duck ELOVL2. Identity/similarity shading was based on the Gonnet series matrix produced by ClustalX where primary black shading indicates identical residues and secondary and tertiary gray shading indicates similar residues with an 80% and 60% cut off, respectively. Seven predicted transmembrane-spanning domains were predicted by using the HMMTOP transmembrane topology prediction server (version 2.0) and are shown with a solid-lined box. The conserved cysteine in all ELOVL2 sequences is indicated with an arrow. ELOVL, elongation of very long-chain FAs.
reason for the lack of DPA elongation activity by duck and turkey ELOVL5 enzymes was not elucidated simply by comparison of the predicted proteins with the chicken ELOVL5 predicted protein. Although there were 2 residue differences between the chicken and turkey ELOVL5 predicted proteins and 4 residue differences between the chicken and duck ELOVL5 predicted proteins, we were unable to identify a particular residue or combination of residues in the chicken ELOVL5, which may be responsible for DPA elongation activity. All 3 bird ELOVL5 predicted proteins have a tryptophan at position 231, which was shown in rats to confer EPA to DPA elongation activity only, not further elongation of DPA to 24:5n–3 (22). The conserved tryptophan in the bird ELOVL5 proteins is in the extracellular loop between the sixth and seventh predicted transmembrane-spanning domains, but the conserved tryptophan in the rat ELOVL5 is within the seventh predicted transmembrane-spanning domain (22). The molecular mechanism behind the ability of chicken ELOVL5 to elongate DPA to 24:5n–3 remains unknown.

Although chicken or turkey ELOVL2 had activity only with C20 and C22 PUFAs, duck ELOVL2 activity was broader with elongation of C18 as well as C20 and C22 PUFAs (3). EPA dose-response curves indicate that the duck ELOVL2 was the most efficient poultry species studied to date to catalyze the sequential elongation reaction EPA to DPA to 24:5n–3 at low EPA concentrations (3). However, duck ELOVL2 conversion of EPA or DPA to 24:5n–3 was saturated at low exogenous substrate concentrations and by using EPA or DPA as a substrate resulted in similar amounts of 24:5n–3 synthesized. The saturation of 24:5n–3 synthesis from EPA or DPA is a characteristic of the chicken ELOVL2 also (3). The overall ability of the duck and chicken ELOVL2 to synthesize 24:5n–3 was most similar, but their predicted proteins were least similar to each other when comparing duck, chicken, and turkey ELOVL2.

FA synthesis in birds occurs mainly in the liver (23). The effect of dietary ALA on liver n–3 LCPUFAs was investigated in chickens and turkeys. In chickens, increasing dietary ALA was seen to significantly increase liver phospholipid EPA, DPA, and DHA (24), or liver phospholipid DHA only (3). In turkeys, increasing dietary ALA increased liver total lipid EPA but had no effect on DPA or DHA (25). We have shown that the turkey ELOVL2 synthesizes the least amount of 24:5n–3 when either EPA or DPA are supplied as exogenous substrates compared with duck and chicken ELOVL2 (3). Thus, ELOVL2 may be limiting the conversion of dietary ALA to n–3 LCPUFAs in turkeys. To our knowledge, there have not been any dietary ALA studies in ducks. By using the elongase enzyme activities only as a predictor of DHA synthesis, ducks may have a similar ability to chickens to convert increasing dietary ALA to DHA (24), or liver phospholipid DHA only (3). In turkeys, increasing dietary ALA increased liver total lipid EPA but had no effect on DPA or DHA (25). We have shown that the turkey ELOVL2 synthesizes the least amount of 24:5n–3 when either EPA or DPA are supplied as exogenous substrates compared with duck and chicken ELOVL2 (3). Thus, ELOVL2 may be limiting the conversion of dietary ALA to n–3 LCPUFAs in turkeys. To our knowledge, there have not been any dietary ALA studies in ducks. By using the elongase enzyme activities only as a predictor of DHA synthesis, ducks may have a similar ability to chickens to convert increasing dietary ALA to DHA (3,24).

Poultry production is growing in both developing and developed countries, and poultry pricing relative to other meats is competitive (10). These factors are important when considering if poultry could be a population source of EPA and DHA. Our study has provided insight into the capacity of chicken, turkey, and duck to be a source of EPA and DHA, which is not derived from fish. Further examination into the fatty acyl desaturation enzymes involved in the chicken and duck FA synthesis pathways is warranted.

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References


