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ORIGINAL ARTICLE

Genome-Wide Identification, Characterization, and Nutrigenomics of *ELOVL* Genes in Laying Hens and Their Responses to Various Levels of Dietary Curcumin

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ABSTRACT

We investigated molecular characteristics and tissue distribution of elongation of very long chain fatty acids (*ELOVL*) genes in the chicken (*Gallus gallus*) genome. The research specifically examines the expression levels of these genes in chickens fed diets enriched with varying concentrations of curcumin. The aim is to uncover the potential roles and functions of *ELOVL* genes in the metabolism of fatty acids (FAs) in this species. In the experimental design, Hy-Line Brown commercial laying hens were selected for a feeding trial lasting 10 weeks. During this period, the hens were fed diets supplemented with curcumin at 100, 200, 300, and 400 mg/kg. The results revealed several critical insights: (1) It was confirmed that the *ELOVL* genes in *Gallus gallus* are orthologues of those found in vertebrates, indicating a shared evolutionary lineage; (2) There were noteworthy differences in how *ELOVL* genes, except *ELOVL1* and *ELOVL7*, were distributed across various tissues and how they responded to dietary curcumin, prompting the need for comprehensive promoter analyses to better understand their functions in chickens; (3) The addition of curcumin to the diet did not increase omega-3 FAs in egg yolk; and (4) Dietary curcumin modulated *ELOVL* mRNA transcription in *Gallus gallus*. However, the intricate and multifaceted nature of FA metabolism presents challenges to fully grasping the implications of these results, highlighting the necessity for further in-depth investigations in this area.

1 | Introduction

About one-third of a chicken egg's overall nutrient composition consists of lipids. These are crucial for nutrition as they provide ω-3 fatty acids (ω-3 FAs), which are essential for healthy growth, development, and cognitive function during early life (Çakmakçı and Kahyaoğlu 2012). Chicken eggs are a source of various nutrients required by the human body in appropriate amounts and proportions, highlighting the importance of lipids. Since mammals have a limited ability, if any, to produce ω-3 FAs, they must acquire these FAs through their diets. A typical chicken egg contains between 30 mg and 200 mg of ω-3, and the ω-3 FA levels can be significantly enhanced by providing the chickens with a diet rich in ω-3 FAs (Yalcin and Unal 2010; Dalle Zotte et al. 2015; Ehr et al. 2017; Kralik et al. 2021).

Chickens excel compared to many other species in converting linoleic acid (18:2n-6; LA) into arachidonic acid (20:4n-6; ARA) through elongation and desaturation activities. Although chickens do not synthesize eicosapentaenoic acid (20:5n-3; EPA) and docosahexaenoic acid (22:6n-3; DHA) from α-linolenic acid (18:3n-3; ALA) with high efficiency, EPA and DHA content in the chicken meat and eggs can be increased by EPA- and DHA-rich diets (Gregory et al. 2013). This is attributed to

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the enzymatic actions of the *ELOVL* and fatty acid desaturase (*FADS*) gene families that facilitate the elongation and desaturation processes.

The *ELOVL* gene family consists of seven isoforms (*ELOVL1–7*), which are important regulators of substrate specificity, tissue distribution, and regulation, as well as overall FA elongation and cellular lipid composition (Ma et al. 2017). Each member of the *ELOVL* gene family has distinct functions. For example, *ELOVL6* is involved in de novo lipogenesis in chicken liver (Claire D'Andre et al. 2013). It encodes the *ELOVL6* enzyme, which regulates the final step of the endogenous saturated fatty acids (SFAs) synthesis. The *ELOVL2*, *ELOVL4*, and *ELOVL5* genes are involved in the elongation of long-chain (LC) FAs (Jakobsson et al. 2006). *ELOVL5* is responsible for the hepatic synthesis of LC-polyunsaturated fatty acids (LC-PUFAs) (Wang et al. 2014; Feng et al. 2020). In addition, this gene plays an important role in the biosynthesis of ω-3 PUFA, including EPA and DHA, as well as ω-6 PUFA (Fu et al. 2018). It has been reported that *ELOVL5* can be extended to tetracosapentaenoic acid (24:5n-3; DPA) in chickens, unlike other poultry, such as ducks and turkeys. Therefore, it has been suggested that chicken may be a dietary source of EPA and DHA, like fish.

Numerous investigations have been conducted to enhance the levels of EPA and DHA in eggs (Dalle Zotte et al. 2015; Ehr et al. 2017; Kralik et al. 2021). Studies indicate that certain biologically active substances incorporated into the diet may promote the conversion of ALA into DHA by stimulating ELOVL enzymes (Fujiyama-Fujiwara et al. 1992; Wu et al. 2015). The utilization of these active compounds can lead to improved yield and product quality, such as curcumin.

Curcumin, recognized as the "golden spice," is derived from the roots of the turmeric plant (*Curcuma longa*), which is cultivated in India and other regions of South Asia. This yellow powder serves not only as a food colorant and flavoring but also functions as a medicinal compound (Moghaddam et al. 2018). Within turmeric, there are several curcuminoids, including curcumin (the most biologically active), demethoxycurcumin, bisdemethoxycurcumin, and cyclocurcumin (Shahrajabian and Sun 2023). Notably, curcumin constitutes about 77%–80% of the total curcuminoid content (Alves et al. 2019). The properties of curcumin, such as increasing DHA in the brain and liver (by enhancing *ELOVL2*), increasing enzymes involved in the DHA synthesis pathway, and reducing anxiety-like behavior, have been identified (Wu et al. 2015). Some researchers have reported that the number of studies investigating the conversion of ALA to EPA and DHA at the molecular level in laying hens is quite limited and that *ELOVL* genes play key roles in elongation and desaturation processes in living organisms (Nakamura and Nara 2004; Lattka et al. 2010; Zhang et al. 2016).

For these reasons, we identified and characterized *ELOVL* genes in the chicken genome for the first time to better understand their functions in FA metabolism in chickens. We also investigated the effects of diets supplemented with various levels of curcumin on hen performance, egg quality characteristics, the FA composition of egg yolk, and the expression levels of the *ELOVL* genes.

2 | Material and Methods

2.1 | Animals, Diets, and Sampling

A total of 40 Hy-Line Brown commercial laying hens, aged 68 weeks, were used in this study. Approval for conducting the study was obtained from the Atatürk University Animal Experiments Local Ethics Committee (2022/59). The hens were divided into five groups of eight. The first group received a basal diet (C0), while the other groups were given diets supplemented with 100 (C100), 200 (C200), 300 (C300), and 400 (C400) mg/kg of curcumin, added to the basal diet. The C0 diet used in the experiment was sourced from a commercial feed mill (Table 1), and the curcumin (99% purity) was acquired from Grenera

TABLE 1 | Composition of basal diet used in the trial (%).

Feed raw materials	Raw material rates
Corn (CP %85)	62.99
Soybean meal (CP %45)	16.57
Corn gluten (CP %60)	7.98
Limestone	10.18
DCP (P %18)	1.44
Vitamin-Mineral mixture[a]	0.25
NaCl	0.22
Sodium bicarbonate	0.16
L-Lysine	0.11
DL-Methionine	0.10
Calculated composition (%)	
Dry matter	88.41
Crude protein	16.52
Crude fat	2.20
Crude ash	12.07
Crude cellulose	2.78
D-Methtionine	0.38
Methionine	0.40
Lysine	0.76
ME (kcal/kg)	2726
Chemical analyses (%)	
Dry matter	88.78
Crude protein	17.02
Crude fat	2.43
Crude ash	11.74
Crude cellulose	3.18

[a]Per kilogram of diet: 12,000 IU vitamin A; 2,500 IU vitamin D3; 30 IU vitamin E; 4 mg vitamin K3; 3 mg vitamin B1; 6 mg vitamin B2; 30 mg niacin; 10 mg calcium D-pantothenate; 5 mg vitamin B6; 0.015 mg vitamin B12; 1 mg folic acid; 0.050 mg D-biotin; 50 mg vitamin C; 300 mg choline chloride; 80 mg manganese; 60 mg iron; 60 mg zinc; 5 mg copper; 0.5 mg cobalt; 2 mg iodine; 0.15 mg selenium.

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Nutrients Pvt. Ltd. Throughout the ten-week experiment, feed and water were provided *ad libitum* to the individually housed hens. Following the conclusion of the feeding trial, the hens were euthanized, and approximately 1 g of liver was collected ($n = 3$). This sample was placed into 2 mL DNase/RNase-free tubes along with 1 mfL of RNAlater and stored at −20°C overnight. Subsequently, it was kept at −80°C until further analysis. Samples from various tissues, including the liver, small intestine, chest muscle, brain, heart, eyes, adipose tissue, gizzard, kidney, skin, ovary, spleen, lung, peel stomach, and leg muscle were obtained from the chickens ($n = 3$) to investigate the tissue-specific distribution of *ELOVL* genes.

2.2 | Determination of General Performance Characteristics

Feed intake, egg weight, egg mass, egg production, and feed conversion ratio were measured every 2 weeks. Quality criteria such as shell thickness, shell strength, Haugh unit, egg yolk color, and the proportions of egg white, yolk, and shell were also assessed through these fortnightly measurements.

2.3 | Determination of Yolk Fatty Acid Composition

The extraction of total lipids from both diets and egg yolks was performed following the methodology established by Folch et al. (1957) at the end of the experiment. To create a homogenate, six samples from each treatment group and diet were combined and ground together. The preparation of fatty acid methyl esters (FAMEs) was conducted using the method described by Metcalfe and Schmitz (1961). These FAMEs were analyzed through gas chromatography, employing a DB-23 capillary column with specifications of 60 m length, 0.25 mm inner diameter, and a film thickness of 0.25 μm. The initial temperature for the ejector and detector was set at 190°C for 35 min and then was raised to 220°C at a rate of 30°C per minute, holding this temperature for an additional 5 min. Hydrogen was used as the carrier gas at a flow rate of 2 mL per minute, with a fixed split ratio of 30:1. Individual fatty acids (FAs) were identified by comparing their retention times to those of a standard FA mixture (Supelco 37 component FAME mix). The quantification of each fatty acid was based on the peak area of the internal standard C19:0 (Sigma, USA) and expressed as a percentage of the total FAMEs.

2.4 | Identification of *ELOVL* Genes

The sequences of mRNA for the chicken *ELOVL1* to *ELOVL7* genes were determined by utilizing the polypeptide sequences of the corresponding zebrafish (*Danio rerio*) Elovls as queries in a specific BLASTP search within the Ensembl genome database (http://useast.ensembl.org/Multi/Tools/Blast?db=core). Additionally, a second tBLASTn search was performed at the National Center for Biotechnology Information (NCBI) (http://blast.ncbi.nlm.nih.gov) with the ELOVL polypeptide sequences to obtain Expressed Sequence Tags (ESTs) for each *ELOVL* gene. However, no ESTs for *ELOVL1*, *ELOVL2*, *ELOVL3*, *ELOVL4*, *ELOVL5*, *ELOVL6*, or *ELOVL7* in chickens were identified in the NCBI EST database (release: 248). As a result, reverse transcription (RT)-PCR was conducted to synthesize the mRNAs for *ELOVL1* through *ELOVL7*, followed by sequencing. The newly obtained sequences were aligned with the genomic sequences of *ELOVL* from Ensembl to verify their exon/intron structures. The molecular weights (MWs) and theoretical isoelectric points (p*I*) of chicken ELOVL proteins were computed with the Protparam software (https://web.expasy.org/protparam/) (Gasteiger et al. 2005). The identification of the ELO domain (Pfam: PF01151) was carried out using the HMMER software, version 3.3 (https://www.ebi.ac.uk/Tools/hmmer/) (Potter et al. 2018). The SignalP-6.0 server was used to determine signal peptide cleavage of ELOVL polypeptides (https://services.healthtech.dtu.dk/services/SignalP-6.0/) (Teufel et al. 2022). The putative TATA boxes and transcription initiation sites were determined using the SoftBerry algorithm (http://www.softberry.com/), and putative polyadenylation signals [poly(A) tail] were calculated at http://www.fruitfly.org/seq_tools/promoter.html (Reese 2001).

2.5 | Orthology of ELOVL Amino Acids

The relationships of orthologous chicken *ELOVL* genes were established through individual BLAST searches, utilizing each putative sequence as a query via the OrthoInspector database (https://www.lbgi.fr/orthoinspectorv3/; Nevers et al. 2019). The MAFFT program was employed for multiple alignments of ELOVL/Elovl amino acid sequences (Katoh et al. 2019). These alignments were reexamined with trimAl to enhance the bootstrap values by eliminating poorly aligned sections (Capella-Gutierrez et al. 2009). A Bayesian phylogenetic tree, created with NGPhlogeny.fr (https://ngphylogeny.fr/; Lemoine et al. 2019), illustrated the phylogenetic relationships among chicken ELOVLs and ELOVLs/Elovls from other vertebrates, comprising 10^6 generations, a sampling frequency of 100, and a burn-in of 10,000. A neighbor-joining (NJ) phylogenetic tree was also generated with 1000 bootstrap replicates using MEGA5 software to allow for comparison of the two phylogenetic trees (Tamura et al. 2011). To assess sequence identity and similarity, the amino acid sequences of chicken *ELOVL* genes were examined against the amino acid sequences of other vertebrates with the BLOSUM62 (Gromiha 2010). Conserved gene synteny of the *ELOVL* genes was analyzed in relation to the *ELOVL*/*elovl* genes from *D. rerio* and humans (*Homo sapiens*) through the Genomicus online database (https://www.genomicus.bio.ens.psl.eu/; Nguyen et al. 2018). All the protein sequences used in this study were supplied in File S1.

2.6 | Total RNA Extraction and Reverse Transcription

Total RNA was extracted from all examined tissues using TRIzol reagent (Invitrogen), with the integrity of the resulting RNA confirmed through 2% agarose gel electrophoresis. The amount of RNA was quantified at 260 nm with a spectrophotometer. To eliminate genomic DNA from the RNA, the DNAse I kit (Life Technologies) was employed. The synthesis of the first-strand cDNA was conducted using an Omniscript RT kit (Qiagen), incorporating two micrograms of total RNA from each tissue sample. The RT was applied at 37°C for 60 min using a PCR machine.

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2.7 | Tissue-Specific Transcription and Nutrigenomics of Chicken *ELOVL* Genes

The quantitative polymerase chain reaction (qPCR) analysis was carried out with the SYBR Green PCR kit (Qiagen). Tissue-specific transcription of *ELOVL* genes was determined in the liver, small intestine, chest muscle, brain, heart, eyes, adipose tissue, gizzard, kidney, skin, ovary, spleen, lung, peel stomach, and leg muscle from chickens fed the control diet ($n = 3$). The conditions for qPCR included an initial denaturation phase at 95°C for 15 min, followed by 40 cycles of denaturation at 95°C for 20 s, annealing for 30 s, elongation at 72°C for 1 min, and a final elongation step at 72°C for 5 min. Negative controls for the qPCR analysis of each *ELOVL* gene were conducted using template-free tubes. PCR and qPCR primers, as listed in Table 2, were designed using NCBI Primer-BLAST (http://www.ncbi.nlm.nih.gov/tools/primerblast/). The PCR product standard was used to generate a standard curve. Amplification factors for both the reference and target genes were determined using the slope values obtained from qPCRs, with an amplification factor of 2 corresponding to 100% efficiency for each gene. The differences in hepatic mRNA expression in laying hens fed diets containing various levels of curcumin were assessed using the $2^{-\Delta\Delta Ct}$ method described by Livak and Schmittgen (2001). *ELOVL* mRNA expression levels were normalized by calculating the ratio of their copy number to that of the reference gene, TATA-box binding protein (ENSGALG00010007866; *TBP*) (Na et al. 2021). Specific primers that span the exon-exon splice junctions for *ELOVL1*, *ELOVL2*, *ELOVL3*, *ELOVL4*, *ELOVL5*, *ELOVL6*, and *ELOVL7* were designed using the NCBI-Primer BLAST tool.

2.8 | Statistical Analysis

The data obtained at the end of the research were analyzed using the General Linear Model procedure and the SPSS package program. The significance of the means found to be significant between the groups was determined by Duncan's multiple comparison test.

3 | Results and Discussion

3.1 | Performance Traits

Our investigation revealed that the addition of varying amounts of curcumin to the diet did not notably affect ($p > 0.05$) feed intake, egg weight, egg production, or feed conversion rates (FCR) in laying hens (Table 3), which is consistent with previous studies (Radwan et al. 2008; Moorthy et al. 2009; Park et al. 2012; Hassan 2016; Zadeh et al. 2022; Li et al. 2024).

3.2 | Egg Internal and External Quality Criteria

The C100 group exhibited the highest Haugh Unit value ($p < 0.019$), likely attributable to the mineral and moisture content of albumin, along with the antioxidant properties of curcumin (Park et al. 2012; Li et al. 2024). Notable differences emerged among the groups concerning the ratios of egg white to yolk, with the C300 group displaying the peak white ratio and the lowest yolk ratio ($p < 0.01$; Table 4). Nevertheless, Li et al. (2024) indicated that the inclusion of curcumin as a

TABLE 2 | PCR and qPCR primers.

Gene	Primers (5′-3′)	Tm (°C)	Amplicon length (bp)	Primer efficiency (%)	r^2
ELOVL1	F: CCCTCAGGATGGTCAGTGTT	60.0			
	R: CTCCAGGGCAGAACAGAGTG		142	98.4	0.99
ELOVL2	F: CTGACATCCGGGTAGCCAAG	59.0			
	R: TCGTGGCATGGTGATACACA		135	99.0	0.99
ELOVL3	F: GGCCTTGTTCAGTGCCATT	58.0			
	R: CCCAGTTCCAGGACTTTGCT		165	98.7	0.97
ELOVL4	F: TGGAGCATCCGAGACAAACG	59.0			
	R: TGGCAGACATAGCTGTACCC		245		
ELOVL5	F: TGGACCCAGAGATCCCAGA	59.0		99.2	0.98
	R: GCCCCTGCATGAGAATGGTT		142		
ELOVL6	F: GCTGTCTTCAGTATATTCGGTGC	60.0		97.9	0.98
	R: CCAGCCACCATGTCCTTGTA		275		
ELOVL7	F: ATCAAAGATGCCGATCCGAG	58.0		99.1	0.99
	R: TTCTTAGGGCTGTAGGCGAC		286		
TBP	F: CAGGAGCAAAAAGCGAGGAAC	58.5		98.9	0.98
	R: TGCTGGTGTGTGAGTACCAA		172		

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dietary additive did not influence the weight of the yolks. A decrease in the shell ratio was observed in both the C100 and C300 groups ($p < 0.030$). Some studies suggest that curcumin might assist in preserving shell ratio due to its properties, which include neutralizing free radicals, lowering oxidative stress, offering anti-inflammatory benefits, mitigating inflammation-related damage, and providing cellular protection (Aggarwal and Harikumar 2009; Gupta et al. 2011). Our findings also indicated that the inclusion of curcumin in diets led to a reduction in shell thickness in all treatment groups apart from C200 in comparison to the control group. Conversely, Liu et al. (2020) reported that varying concentrations of curcumin added to the diets of hens under heat stress significantly enhanced shell thickness. Previous research has established that the strength of a standard eggshell ranges from 2.7 to 4.9 kg/cm^2 (Voisey and MacDonald 1978). In this study, while curcumin was associated with diminished shell strength relative to the C0 group, the shell strength measurements remained within the standards outlined by Voisey and MacDonald (1978).

TABLE 3 | Effect of diets containing different levels of curcumin on performance.

Groups	Feed intake (g)	Egg weight (g)	Egg production (%)	Feed covertion ratio (g/g)
C0	115.03	64.14ab	87.10	2.10
C100	109.55	62.92b	86.72	2.05
C200	117.40	64.73ab	90.00	2.04
C300	116.14	66.10a	87.67	2.04
C400	112.33	63.08b	91.76	1.96
SE	1.133	0.354	0.795	0.025
P	NS	0.03*	NS	NS

Note: C0: Group without added curcumin, C100: 100 mg curcumin added group, C200: 200 mg curcumin added group, C300: 300 mg curcumin added group, C400: 400 mg curcumin added group, a,b: Differences between group means shown with different letters in the same column are significant.
Abbreviations: NS: not significant; SE: standard error.
*$p < 0.05$.
**$p < 0.01$.

3.3 | Fatty Acid Composition of Egg Yolk

The study's conclusion revealed that the inclusion of curcumin had a significant impact on the FA composition of egg yolk, with the exceptions of myristic and palmitoleic acids. Palmitic acid levels rose alongside curcumin concentration, peaking in the C300 and C400 groups ($p < 0.01$). Meanwhile, curcumin supplementation notably reduced stearic acid levels, with the C100 group exhibiting the lowest amount ($p = 0.003$). Additionally, oleic acid levels were also lowest in the C100 group. As indicated in Table 5, there were highly significant differences in LA and ALA levels among the groups, with the C100 group showing the highest values for both FAs ($p = 0.001$, $p = 0.000$). However, rising levels of curcumin led to a marked decrease in the ratios of these FAs. A significant difference was observed in ARA levels, with the C400 group presenting the lowest value and the C300 group the highest ($p = 0.000$). The C400 group had the lowest ratio of DHA ($p = 0.001$). Although the EPA levels differed significantly between groups ($p = 0.001$), the addition of curcumin at 100 and 300 mg/kg resulted in a reduced EPA ratio.

Many problems such as anxiety, Alzheimer's disease, major depressive disorder, and attention deficit disorder are associated with DHA deficiency (Astarita et al. 2010; Bhatia et al. 2011; Chen and Su 2013; McNamara et al. 2013). Therefore, ω-3 PUFAs play a critical role in brain health and the prevention of cognitive disorders in general. In this study, LA levels were quite low in the C200 group, while DHA levels were the highest. It is thought that the addition of 200 mg/kg of curcumin to the diet promotes the conversion of LA to DHA. It has been

TABLE 4 | Effect of diets containing different levels of curcumin on egg quality criteria.

Groups	Egg yellow color	Haugh unit	Albumen (%)	Egg yolk (%)	Shell ratio (%)	Shell thickness (μm)	Shell breaking strength (kg/cm^2)
C0	9.68	78.12bc	59.15c	29.04a	11.81a	0.393ab	4.30a
C100	9.61	84.61a	61.14ab	27.61ab	11.26b	0.376c	3.69bc
C200	9.79	82.60ab	60.04bc	28.06ab	11.90a	0.399a	4.09ab
C300	9.69	79.99abc	62.22a	26.57b	11.22b	0.372c	3.77bc
C400	9.40	77.29c	59.53bc	28.92a	11.54ab	0.383bc	3.51c
SE	0.083	0.793	0.289	0.251	0.085	0.002	0.071
P	NS	0.02*	0.00**	0.01**	0.03*	0.00**	0.00**

Note: C0: Group without added curcumin, C100: 100 mg curcumin added group, C200: 200 mg curcumin added group, C300: 300 mg curcumin added group, C400: 400 mg curcumin added group, a,b: Differences between group means shown with different letters in the same column are significant.
Abbreviations: ns: not significant, SE: standard error.
*$p < 0.05$.
**$p < 0.01$.

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TABLE 5 | Effect of diets containing different levels of curcumin on the fatty acid composition of egg yolk (%).

Fatty acids	C0	C100	C200	C300	C400	SE	P
14:0	2.02	2.12	1.85	1.86	1.79	0.044	ns
16:0	22.40bc	21.79c	22.93ab	23.27a	23.15a	0.139	0.002**
18:0	8.37a	7.64c	7.89bc	8.17ab	7.88bc	0.049	ns
∑SFA	32.80a	31.56b	32.68a	33.31a	32.83a	0.066	0.003**
16:1n-7	2.21	2.28	2.42	2.41	2.60	0.292	0.001**
18:1n-9	40.05ab	37.79c	39.33b	41.22a	40.47ab	0.373	0.000**
∑MUFA	42.26bc	40.07c	41.76b	43.64a	43.07ab	0.028	0.000**
18:3n-3	1.05b	1.10a	0.86c	0.78d	0.85c	0.026	0.023*
20:5n-3	0.31a	0.26b	0.27b	0.25b	0.30a	0.017	0.013*
22:6n-3	1.15c	1.15b	1.18a	1.13d	1.02e	0.006	0.001**
∑ω-3 PUFA	2.51a	2.50a	2.30b	2.16c	2.17c	0.157	0.011*
18:2n-6	17.74b	20.98a	18.67b	16.05c	17.34bc	0.303	0.001**
20:4n-6	2.07ab	2.15ab	2.10ab	2.21a	1.97b	0.399	0.000**
∑ω-6 PUFA	19.82bc	23.13a	20.77b	18.26d	19.31cd	0.037	0.000**
∑PUFA	22.33bc	25.64a	23.08b	20.42d	21.49cd	0.371	0.000**
∑ω-3 PUFA/∑ω-6 PUFA	0.13a	0.11c	0.11c	0.12b	0.11c	0.001	0.000**
DHA/EPA	3.74c	4.50a	4.10b	4.56a	3.35d	0.10	0.000**

Note: C0: Group without added curcumin, C100: 100 mg curcumin added group, C200: 200 mg curcumin added group, C300: 300 mg curcumin added group, C400: 400 mg curcumin added group, a,b,c,d,e: Differences between group means shown with different letters in the same raw are significant.
Abbreviations: ns: not significant, SE: standard error.
*$p < 0.05$.
**$p < 0.01$.

reported that the *ELOVL4* and *ELOVL5* genes are responsible for the elongation of PUFA (Nakamura and Nara 2004; Zhang et al. 2016). It was found that the *ELOVL5* gene expression was significantly higher in the C200 group than in the C0 group (Figure 1). Similarly, DHA levels in the liver of rats fed diets containing ALA + 250 ppm curcumin were found to be the highest compared to other groups. The same researchers reported that curcumin may increase the conversion of ALA to DHA in vivo, resulting in increased DHA in the liver, which might be an explanation for the highest DHA content in the C200 group. The EPA level was lower in the C200 group than in the C0 group. As EPA is converted to DHA (Li et al. 2010; Morais et al. 2012), the lowest level of EPA and the highest level of DHA in the C200 group can be explained by the conversion of EPA to DHA.

3.4 | *ELOVL* Genes in the Chicken Genome

We identified seven *ELOVL* genes in the chicken genome based on *in silico* analyses and subsequently uploaded their open reading frame (ORF) sequences to the NCBI genome database. The accession numbers for these cDNAs are as follows: *ELOVL1* (873 bp) is PP601341, *ELOVL2* (894 bp) is PP601342, *ELOVL3* (759 bp) is PP601343, *ELOVL4* (945 bp) is PP601344, *ELOVL5* (888 bp) is PP601345, *ELOVL6* (798 bp) is PP601346, and *ELOVL7* (840 bp) is PP601347. Correspondingly, the ORFs translate into 290, 297, 252, 314, 295, 265, and 279 amino acids.

The *ELOVL* genes comprise varying numbers of exons: *ELOVL1* has 7, *ELOVL2* has 8, *ELOVL3* has 4, *ELOVL4* has 6, *ELOVL5* has 7, *ELOVL6* has 4, and *ELOVL7* has 7. Consequently, there is a lack of a conserved gene structure among the *ELOVL* genes in chickens. All characterized *ELOVL* genes adhered to the GT-AG intron splicing motif (Mount 1982) (File S2). The putative TATA boxes, transcription initiation sites, and poly(A) tails of *ELOVL* genes were also provided in File S2.

The MWs and theoretical p*I* values of ELOVL amino acids varied, ranging from 29.92 kDa for ELOVL3 to 36.76 kDa for ELOVL4, and from 9.18 for ELOVL7 to 9.65 for ELOVL4, respectively. No signal peptide cleavage was determined in FADS polypeptides, indicating they could be non-secretory proteins (Ding et al. 2017). The HXXHH motif along with the ELO domain (Pfam: PF01151) is highly conserved across ELOVL polypeptides (Liu et al. 2024). All chicken ELOVL proteins examined included both the PF01151 domain and the HXXHH motif (File S3).

3.5 | Alignment of Chicken ELOVL Polypeptides With Polypeptide Sequences of ELOVL/Elovl From Various Vertebrate Species

The ELOVL protein sequences in chickens showed the highest levels of identity and similarity when compared to ELOVL/Elovl

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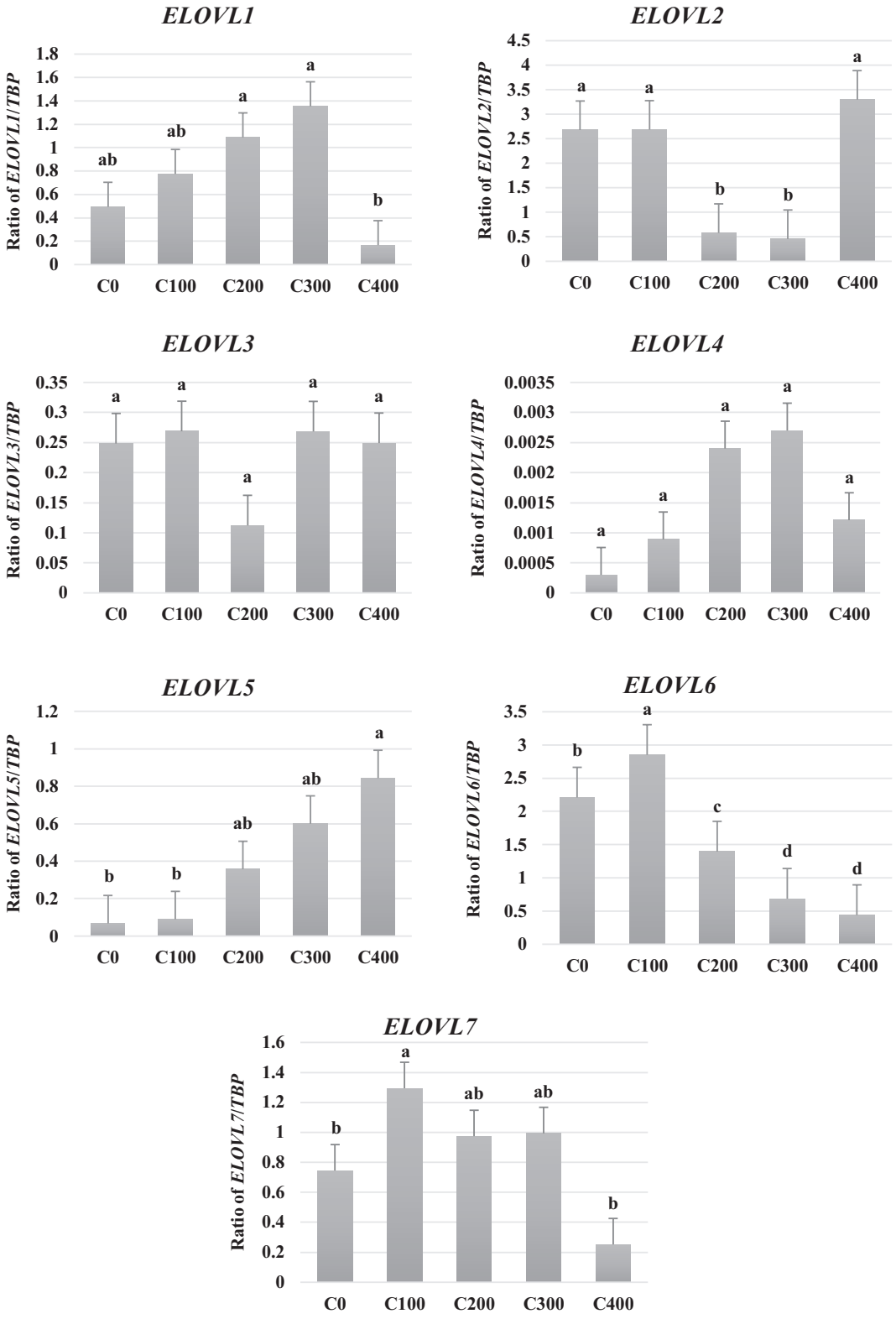

FIGURE 1 | mRNA expression levels in the livers of laying hens fed diets containing various levels of curcumin.

orthologs found in other avian species (File S4). A gradual decline in sequence identity and similarity was observed between the chicken ELOVL sequences and their orthologous counterparts in fish and mammals. This decline continued further with non-orthologous sequences from these same groups, providing additional evidence that the chicken genome comprises seven *ELOVL* genes.

3.6 | The Phylogenetic Relationships of Chicken ELOVL Proteins in Comparison to Those From Other Vertebrate Species

A Bayesian phylogenetic tree was constructed using ELOVL amino acid sequences from a variety of birds, fishes, and mammals to explore the evolutionary relationships of the putative

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ELOVL proteins in chickens and their orthologous counterparts across different vertebrates (Figure 2). The clustering of chicken ELOVL proteins was observed in specific clades alongside ELOVL/Elovl proteins from other vertebrate species. Moreover, a NJ method applied to the same vertebrate ELOVL/Elovl protein sequences produced a phylogenetic tree with a topology similar to the Bayesian analysis (File S5). Both phylogenetic analyses reveal that ELOVL polypeptides can be categorized into three primary branches. Branch 1 includes ELOVL1 and ELOVL7, branch 2 comprises ELOVL3 and ELOVL6, while branch 3 contains ELOVL2, ELOVL4, and ELOVL5. These phylogenetic evaluations aligned with the findings from BLAST database searches that pinpointed the amino acid-encoding *ELOVL* genes, as well as the protein sequence alignments that demonstrated levels of sequence identity and similarity, confirming the presence of seven *ELOVL* genes within the chicken genome, which is the same as in mammals (Liu et al. 2024).

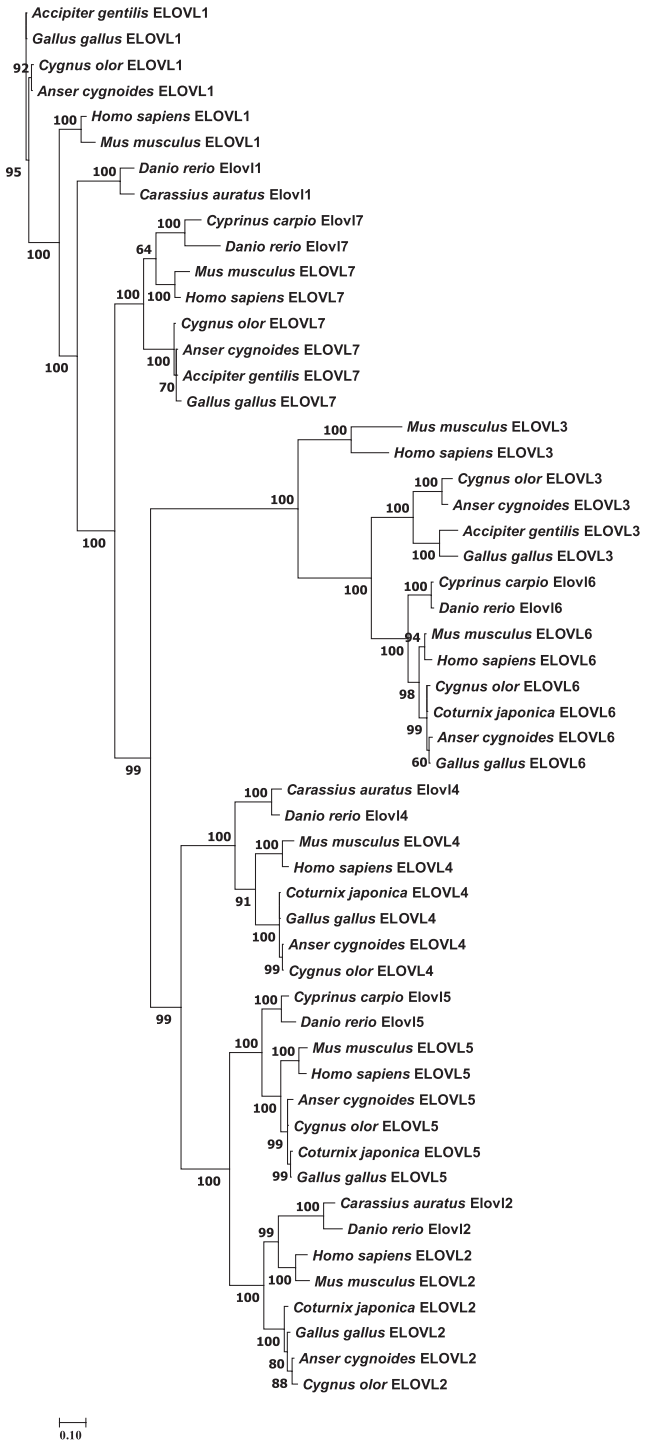

FIGURE 2 | A Bayesian phylogenetic tree illustrating the phylogenetic relationships among chicken ELOVLs and ELOVL/Elovl from other vertebrates.

3.7 | Conserved Gene Synteny Analysis of Chicken *ELOVL* Genes With *ELOVL*/*elovl* Genes From Zebrafish and Humans

The *ELOVL1*, *ELOVL2*, *ELOVL3*, *ELOVL4*, *ELOVL5*, *ELOVL6*, and *ELOVL7* genes in the chicken genome were located on chromosomes 8, 2, 6, 3, 3, 4, and Z, respectively. Additionally, the conserved synteny shared by the chicken *ELOVL* gene family and the *ELOVL*/*elovl* genes found in zebrafish and humans supports the hypothesis that the chicken *ELOVL* genes are orthologous to those in other vertebrates (File S6). Consequently, *in silico* analyses indicate that the chicken *ELOVL* genes evolved from a shared ancestral gene.

3.8 | Tissue-Specific Transcription of *ELOVL* Genes in the Chicken Genome

According to qPCR analysis, the expression levels of *ELOVL* gene transcripts varied significantly in the liver, small intestine, chest muscle, brain, heart, eyes, adipose, gizzard, kidney, skin, ovaries, spleen, lungs, peel stomach, and leg muscle, with the exception of *ELOVL1* and *ELOVL7* (File S7). Wang et al. (2022) identified 51 single nucleotide polymorphisms (SNPs) within chicken *ELOVL* genes, noting that SNPs were absent only in the *ELOVL1* and *ELOVL7* genes. Consequently, we proposed that *ELOVL1* and *ELOVL7* could retain the original functions of *ELOVL* genes. The highest mRNA expression of *ELOVL2*, *ELOVL3*, *ELOVL4*, *ELOVL5*, and *ELOVL6* was determined in the liver, adipose, skin, peel stomach, and liver, respectively. A similar tissue-specific distribution was reported by Mihelic et al. (2020) for *ELOVL2*, *ELOVL5*, and *ELOVL6* in chickens. Differential transcription of chicken *ELOVL* genes in various tissues suggests that they have different functions due to the accumulation of mutations in their transcriptional control sites after their divergence from the ancestral *ELOVL* gene. At this point, we examined how dietary curcumin influences the transcripts of the chicken *ELOVL* genes, aiming to gain a deeper insight into the role of *ELOVL* genes in the metabolism of FAs in chickens.

3.9 | Nutritional Regulation of the *ELOVL* Genes by Diets Containing Various Levels of Curcumin

The hepatic mRNA expression levels of chicken *ELOVL* genes were significantly influenced by diets containing various levels of curcumin. Both *ELOVL1* and *ELOVL7* genes exhibited a similar response to the curcumin supplemented diets, with the C400 diet leading to a reduction in their transcriptions (Figure 1).

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This observation further suggests that *ELOVL1* and *ELOVL7* may share functional similarities. As previously mentioned, *ELOVL2*, *ELOVL4*, and *ELOVL5* genes cluster in the Bayesian and NJ phylogenetic trees. However, dietary curcumin did not uniformly affect the mRNA expressions of ELOVL genes. The lowest expression of *ELOVL2* mRNA was observed in the C200 and C300 diets, whereas the highest transcription levels of *ELOVL4* were found in the same C200 and C300 diets. Meanwhile, the *ELOVL5* gene showed maximum expression when provided with C200, C300, and C400 diets. Previous reports suggested that *ELOVL* genes show substrate specificity: *ELOVL2*, *ELOVL4*, and *ELOVL5* preferentially utilize PUFAs, while *ELOVL1*, *ELOVL3*, *ELOVL6*, and *ELOVL7* prefer SFAs and MUFAs as their substrates (Monroig et al. 2010). The *ELOVL1* gene exhibited the highest transcription levels in the C200 and C300 groups, while *ELOVL3* showed peak expression in the C0, C100, C300, and C400 groups. For *ELOVL6*, the maximum transcription was observed in the C100 group, and *ELOVL7* demonstrated elevated levels in the C100, C200, and C300 groups (Figure 1). These findings effectively demonstrate that diets supplemented with various levels of curcumin do not influence substrate specificity in chicken *ELOVL* genes.

4 | Conclusions

In our study, we cloned ORFs of seven *ELOVL* genes found in the chicken genome. From our bioinformatics investigations, along with examinations of tissue-specific distribution and the nutritional regulation of *ELOVL* genes, we proposed several conclusions: (1) *ELOVL/elovl* genes in chickens and other vertebrates are orthologous and likely evolved from a common ancestral gene. (2) *ELOVL1* and *ELOVL7* demonstrated similar patterns in tissue-specific transcription; however, their reactions to diets supplemented with curcumin differed. (3) It was determined that the diet enriched with 200 mg/kg curcumin significantly increased the Haugh Unit and DHA ratio in egg yolk. (4) This research is the first to investigate the nutritional regulation of *ELOVL* genes using various curcumin dietary levels in chickens, revealing that the mRNA expression of each *ELOVL* gene responds differently to dietary curcumin. Nevertheless, the intricate nature of FA metabolism complicates a complete understanding of the results, and more detailed investigation is needed.

Author Contributions

Büşra Dumlu: conceptualization, methodology, sampling, experimentation, data curation, writing the original draft, and editing. **Şaziye Canan Bölükbaşi:** conceptualization, methodology, project administration, funding, sampling, experimentation, data curation, writing the original draft, and editing; **Abdulkadir Bayir:** conceptualization, methodology, writing the original draft, and editing.

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Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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Supporting Information

Additional supporting information can be found online in the Supporting Information section. **File S1:** lipd70013-sup-0001-Supplementaryfile1.pdf. **File S2:** lipd70013-sup-0002-Supplementaryfile2.pdf. **File S3:** lipd70013-sup-0003-Supplementaryfile3.pdf. **File S4:** lipd70013-sup-0004-Supplementaryfile4.pdf. **File S5:** lipd70013-sup-0005-Supplementaryfile5.pdf. **File S6:** lipd70013-sup-0006-Supplementaryfile6.pdf. **File S7:** lipd70013-sup-0007-Supplementaryfile7.pdf.

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