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Effect of Vitamin E Supplementation on Deposition and Gene Expression Profiling of Abdominal Fat in Broiler Chickens

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The aim of this study was to study the regulation of abdominal fat deposition by DL-α-tocopherol acetate (vitamin E) in broilers. Diets supplemented with 50 IU vitamin E significantly diminished abdominal fat deposition in broilers at day 35. Transcriptome sequencing results for abdominal fat tissues of the control (FC) and 50 IU vitamin E-supplemented (FT) groups identified 602 differentially expressed genes (DEGs), which were enriched in cellular process, cell and cell part, and binding Gene Ontology terms. Pathway functional analysis revealed that the DEGs were enriched in 42 metabolic pathways. Notably, the most enriched pathway, fatty acid biosynthesis, was found to play a key role in lipid metabolism. Further, the key regulators of lipid metabolism, including fatty acid synthase, acetyl-CoA carboxylase alpha, and acyl-CoA synthetase long-chain family member 1, demonstrated decreased expression following vitamin E supplementation. Herein, we have identified pathways and genes regulated by vitamin E, thereby providing novel insights into the nutrients regulating abdominal fat deposition in broilers.

Key words: abdominal fat deposition, broiler, gene expression, RNA-seq, vitamin E

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Introduction

Vitamin E is an essential micronutrient for humans and animals. There are eight fat-soluble vitamin E compounds (α-, β-, γ-, and δ-tocopherol and α-, β-, γ-, and δ-tocotrienol), with α-tocopherol being one of the most active forms (Gonzalez-Calvo *et al*., 2017). Human and animal diets are commonly fortified with α-tocopherol to meet basal nutritional requirements. Vitamin E plays vital roles in physiological processes, such as fertility, oxidative homeostasis, signal transduction, and gene regulation, and in diseases, such as non-alcoholic fatty liver. Clinical insights regarding the roles of vitamin E have been previously summarized (Galli *et al*., 2017). Recently, the role of vitamin E and its metabolites in regulating cell signaling and gene transcription have received increased attention (Azzi, *et al*., 2018). Moreover, the role of vitamin E in regulating lipid metabolism and abdominal fat deposition has also been investigated (Sun *et al*., 2015). Studies have shown that vitamin E supplementation can prevent human lipid metabolism-related diseases, such as non-alcoholic fatty liver (Oliveira *et al*., 2003; Sato *et al*., 2015) and diabetes (Yan *et al*., 2017; Alkholy *et al*., 2018), and improve insulin sensitivity in these patients (Gray *et al*., 2011). In animals, dietary vitamin E increased the levels of certain polyunsaturated fatty acids in muscle tissue, which led to a higher percentage of these molecules in long-term frozen contained meat (Ferrinho *et al*., 2018). Dietary vitamin E has also been shown to prevent diet-induced lipid accumulation in the liver of guinea pigs (Podszun *et al*., 2014). Moreover, dietary vitamin E supplementation decreased the amount of abdominal fat in broiler chickens (Li *et al*., 2009; Zaboli *et al*., 2013) and the same results were found under conditions of heat stress (Habibian *et al*., 2014).

The regulatory roles of vitamin E on lipid metabolism may

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involve inhibition of adipose cell conversion by regulating signaling pathways and modulating gene transcription. A previous study showed that vitamin E inhibited the adipose conversion of 3T3-L1 cells at micromolar levels (Kawada *et al*., 1900). Further studies indicated that vitamin E induced adiponectin expression in rat adipose tissues and 3T3-L1 cells via a peroxisome proliferator-activated receptor γ-dependent mechanism (Landrier *et al*., 2009). Adiponectin is important in lipid metabolism because it promotes adipocyte differentiation, insulin sensitivity, and lipid accumulation (Fu *et al*., 2005).

Vitamin E can also affect fat deposition by regulating the expression of genes involved in lipid metabolism, such as those involved in lipogenesis, lipolysis, and transport. In broilers, 200 mg/kg dietary vitamin E supplementation increases *PPAR-*β and *H-FABP* expression in the pectoralis (Li *et al*., 2009). In light lambs, short-term α-tocopherol treatment affected *ABCA1*, *LPL*, *APOE*, and *SREBP1* expression in the L. thoracis (LT) muscle and *ABCA1*, *SCARB1*, *LPL*, and *PPARG* in subcutaneous fat (SF) cells; in contrast, *PPARA* expression was upregulated, but only after long-term α-tocopherol treatment (Gonzalez-Calvo *et al*., 2014). A study that compared the gene expression profiles of lambs that received vitamin E (500 mg/kg DL-α-tocopheryl acetate) and controls found that vitamin E supplementation dramatically affected the gene expression profile of SF cells; there was a general upregulation of significant genes compared to the control animals. Vitamin E supplementation caused a downregulation of genes related to intracellular signaling in the LT. Functional analysis of SF cells showed that vitamin E supplementation resulted in an upregulation of lipid, cholesterol, sterol, and steroid biosynthesis genes, and a downregulation of stress response genes (Gonzalez-Calvo *et al*., 2017). However, there have been few studies on the role of vitamin E in abdominal fat. Moreover, the regulatory mechanisms of vitamin E on lipid metabolism are incompletely understood.

In the chicken industry, broilers grown for meat have a fast growth rate; however, they often have excessive deposits of body fat, especially abdominal fat (Zaboli *et al*., 2013; Huang *et al*., 2015). Abdominal fat is highly correlated with total carcass lipids and is used as the main criterion for assessing excess fat deposition in broilers (Chambers *et al*., 2009). Dietary vitamin E (α-tocopherol acetate) supplementation has been shown to significantly decrease abdominal fat content (Li *et al*., 2009; Zaboli *et al*., 2013). Thus, chickens are an ideal model organism for studying abdominal obesity; therefore, we investigated the key genes and metabolic pathways associated with reduced abdominal fat deposits in broilers fed on a diet supplemented with dl-α-tocopherol acetate using RNA sequencing (RNA-seq) to identify pathways that could be targeted to prevent obesity in humans.

Materials and Methods

Animals, Experimental Design, and Diets

This study was conducted in accordance with the Guidelines for Experimental Animals established by the Ministry of Science and Technology (Beijing, China). A total of 240 1-day-old healthy female chicks, which had no significant differences in birth weight between each treatment (42.35± 1.92 g, $P>0.05$), were randomly allocated into five dietary treatments. Each treatment had six replicates and each replicate had eight chicks. The two period (0−21 days and 22−35 days) corn/soybean meal basal diet was provided. The basal diet composition is shown in Table 1. The vitamin E (α-tocopherol) concentrations in the diet were measured as previously described (Li *et al*., 2009). The diet contained 82.4 and 90.1 IU vitamin E in the 0−21 day and 22−35 day periods, respectively. Chickens from the five treatment groups were fed on a basal diet supplemented with 0, 20, 50, 75, and 100 IU dietary DL-α-tocopheryl acetate (vitamin E, 1 mg DL-α-tocopheryl acetate＝1 IU alpha-tocopherol, Xinchang Hebao Biotechnology Co. Ltd, Xinchang, China) over the 35-d trial period. All broilers were raised in stair-step cages with *ad libitum* access to feed and fresh water under the recommended environmental settings according to the normal management practices at the Animal Feeding Room of Longyan University. Broilers were conventionally vaccinated for Marek's disease at birth, Newcastle disease and infectious bronchitis at days 7 and 21, and infectious bursal disease at day 14.

Table 1. **Ingredients and chemical composition of basal diets for broilers[1]**

Item	0 to 21 days	22 to 35 days
Ingredients		
Corn	478.1	534.5
Soybean meal	318.1	281.7
Wheat	80.0	50.0
Soybean oil	51.2	60.8
Corn gluten meal	30.0	30.0
Limestone	12.3	11.0
Dicalcium phosphate	14.6	15.3
DL-Methionine	1.6	1.9
L-Lysine	1.1	1.8
Salt	3.0	3.0
Premix	10.0	10.0
Total	1000	1000
Chemical composition[2]		
ME, MJ/kg	12.98	13.36
CP	205.0	190.0
Calcium	9.0	8.5
Available P	4.5	4.5
Lysine	10.5	10.2
Methionine	4.5	4.5
Vitamin E, IU	82.4	90.1

Note: [1] Provided per kg of premix: Se (Na_2SeO_3) 0.3 mg, Cu ($CuSO_4$)10 mg, Mn ($MnSO_4$) 60 mg, Zn ($ZnSO_4$) 120 mg, Fe ($Fe_2(SO4)_3$) 60 mg, VA 10000 IU, VD_3 3500 IU, VK_3 5000 IU, VB_1 2 mg, VB_2 5 mg, VB_6 4 mg, VB_{12} 0.04 mg, biotin 0.20 mg, folic acid 2.0 mg, niacin 40 mg, calcium pantothenate 20 mg.
[2] Vitmian E is DL-α-tocopheryl acetate, 1 mg DL-α-tocopheryl acetate＝1 IU alpha-tocopherol; the values in nutrient levels of the diet are calculated except VE.

Samples and Measurements

At the end of each trial period, all broilers were weighed after fasting for 12 h. One broiler from each replicate was randomly selected, weighed and killed by stunning and exsanguination after 12 h of fasting. The abdominal fat was weighed as previously described (Li *et al*., 2009; Sun *et al*., 2013), and samples were stored at −20℃ or in liquid nitrogen until further use. Because there were greater abdominal fat weight (AFW) differences between the 50 IU vitamin E group and controls, three abdominal fat tissue samples from the control group (FC) and three from 50 IU vitamin E treatment group (FT) were used for further study.

Before euthanasia, blood was collected from the brachial vein of one broiler from each replicate by venipuncture, and the sera of these broilers were separated by centrifugation at 2,000 rpm for 5 min at 25℃ and frozen at −80℃. Serum α-tocopherol levels were measured using assay kits purchased from Nanjing Jiancheng Bioengineering Institute (Nanjing, China) according to the manufacturer's instructions.

mRNA Library Construction and Sequencing

Total RNA was isolated from abdominal fat tissue using the RNAsimple Total RNA kit (Tiangen Biotech Inc., Beijing, China) in accordance with the manufacturer's instructions. RNA integrity and concentrations were evaluated using an Agilent 2100 Bio-analyzer (Agilent Technologies, Palo Alto, CA, USA), and the RNA was treated with Ambion DNase I (Life Technologies Inc., Carlsbad, CA, USA) following the manufacturer's instructions. The average RNA integrity number (RIN) of each sample was 9.1, indicating sufficient quality for transcriptome sequencing. The mRNA was enriched from total RNA samples using oligo (DT) magnetic beads (Invitrogen, Carlsbad, CA, USA). Purified mRNA was first fragmented using an RNA fragmentation kit (Ambion, Austin, TX, USA), and then first-strand cDNA was synthesized. Subsequently, second-strand cDNAs were synthesized according to the manufacturer's instructions. The cDNA library was assessed on the Agilent 2100 Bioanalyzer and the ABI Step One Plus Real-Time PCR System. Final cDNA libraries were sequenced using the Illumina HiSeq 2500 system.

RNA-seq Data Processing and Analysis

Raw reads of each sample were obtained by Illumina sequencing. Clean reads omitted the low-quality reads and reads with adaptors and unknown bases using SOAPnuke v1.5.2 (Chen *et al*., 2018). Clean reads were mapped to the chicken reference genome using HISAT2 v2.0.4 (Kim *et al*., 2015). The mapping rate of the genes was statistically accurate, suggesting clean reads mapped to the chicken reference genome using Bowtie2 v2.2.5 (Langmead *et al*., 2012); expression levels were calculated by RSEM v1.2.12 (Li *et al*., 2011). A heatmap of all expressed genes was created in R v3.4.3 using the pheatmap package. Differentially expressed genes (DEGs) between the FC and the FT groups were identified using the DESeq2 method (Love *et al*., 2014). Genes with an absolute value of log2 (FT/FC) fold change $\geq$ 2.00 and an adjusted *P*-value $\leq$ 0.05 were considered to be DEGs in this study.

Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG)pathway Analysis

GO analysis for DEGs between the FC and FT groups was performed using Blast2GO, a software package that retrieves GO terms (Götz *et al*., 2008). The significance level of the GO terms was set as false discovery rate-adjusted *P*-values $\leq$ 0.05. The KEGG ontology (KO) enrichment analyses for the DEGs were performed to further elucidate their biological functions. In all tests, *P*-values were calculated using the Benjamini and Hochberg FDR correction test and *P*-values $\leq$ 0.05 were considered to be statistically significant.

Validation of RNA-seq Results using Quantitative Real-time PCR (qPCR)

To validate the repeatability and reproducibility of the gene expression data, quantitative real-time PCR (qPCR) of nine selected DEGs was performed. β-actin was used as a reference gene. All primer sequences were designed using Primer Premier 5 software (Premier Biosoft, Palo Alto, CA, USA). Total RNA was isolated from abdominal fat tissue with the RNAsimple Total RNA kit (Tiangen Biotech, Beijing, China). First-strand cDNA was synthesized from 2 μg total RNA using the Reverse Transcription Kit (Promega, Beijing, China). Power SYBR$^{®Green}$ PCR Master Mix (Applied Biosystems) was used to analyze mRNA levels of selected genes. Next, qPCRs were performed on an ABI 7500 Real-time Detection System (Applied Biosystems). Amplification was performed in a total volume of 20 μL containing 10 μL of 2 ×PCR Master Mix, 100 ng cDNA, 0.5 μL of each primer (10 μmol), and 8.0 μL ddH2O. To ensure similar PCR efficiencies (close to 100%) between target genes and the reference gene (β-actin), primer and cDNA concentrations were optimized as needed. The following PCR conditions were used: 95℃ for 10 mins, followed by 40 cycles of 95℃ for 15 s, 60℃ for 20 s and 72℃ for 32 s. To determine fold changes in gene expression, the comparative CT method was used (Livak *et al*., 2001), calculated as 2^{-Liva}. The results are expressed as the mean fold change in gene expression from triplicate analyses.

Statistical Analysis

The statistical analysis of growth performance, carcass performance, and serum α-tocopherol concentrations between the treatments were performed with one-way analysis of variance (ANOVA) using SPSS, version 19.0 (IBM, Armonk, NY, USA), and the results are expressed as mean and pooled square estimated margin (SEM). Differences were tested at levels of significance of 0.05 and 0.01 with the least significant difference (LSD) multiple comparisons method. Statistical analyses of gene expression levels between the FC and FT groups were performed with independent-sample T test using SPSS v19.0. Differences were tested at a level of significance of 0.05 with the t statistic.

Results

Growth Performance, Fat Deposition, and Serum Vitamin E Content

For the growth performance, only F/G was significantly increased after 5 weeks of 25 IU vitamin E treatment com-

Table 2. **Effect of dietary vitamin E levels on growth performance in broilers**[1]

Item[3]	Vitamin E levels[2]					*P*-value	Pooled SEM
	0 IU	25 IU	50 IU	75 IU	100 IU		
7 days							
ADG (g/day)	14.23	14.79	14.04	14.82	14.70	0.1958	0.05
ADFI (g/day)	18.23	17.50	16.90	17.59	18.08	0.3621	0.19
F/G	1.29	1.18	1.20	1.19	1.23	0.2648	0.00
14 days							
ADG (g/day)	28.37	28.05	27.09	28.67	27.66	0.4281	0.28
ADFI (g/day)	41.13	43.76	39.81	43.52	41.00	0.0535	0.66
F/G	1.51	1.56	1.51	1.52	1.48	0.1653	0.00
21 days							
ADG (g/day)	40.56	39.67	40.46	42.17	39.86	0.4578	0.92
ADFI (g/day)	69.69	66.78	65.73	67.86	65.14	0.5881	3.99
F/G	1.72	1.69	1.63	1.61	1.64	0.5873	0.00
28 days							
ADG (g/day)	61.96	61.92	59.38	59.53	61.45	0.7890	3.20
ADFI (g/day)	92.51	90.1	91.92	96.25	89.86	0.7904	13.46
F/G	1.49	1.46	1.55	1.62	1.46	0.1088	0.00
35 days							
ADG (g/day)	68.04	68.89	73.8	70.46	74.62	0.3458	6.30
ADFI (g/day)	125.88	142.11	140.01	137.43	143.52	0.1066	19.91
F/G	1.85[b]	2.08[a]	1.90[b]	1.95[ab]	1.93[ab]	0.0382	0.00

Note: [1] Data are means and pooled SEM (n=6); different lowercase superscripts in each column indicate significant differences.
[2] Vitmian E is DL-α-tocopheryl acetate, 1 mg DL-α-tocopheryl acetate=1 IU alpha-tocopherol.
[3] ADG, average daily gain; ADFI, average daily feed intake; F/G, feed/gain ratio.

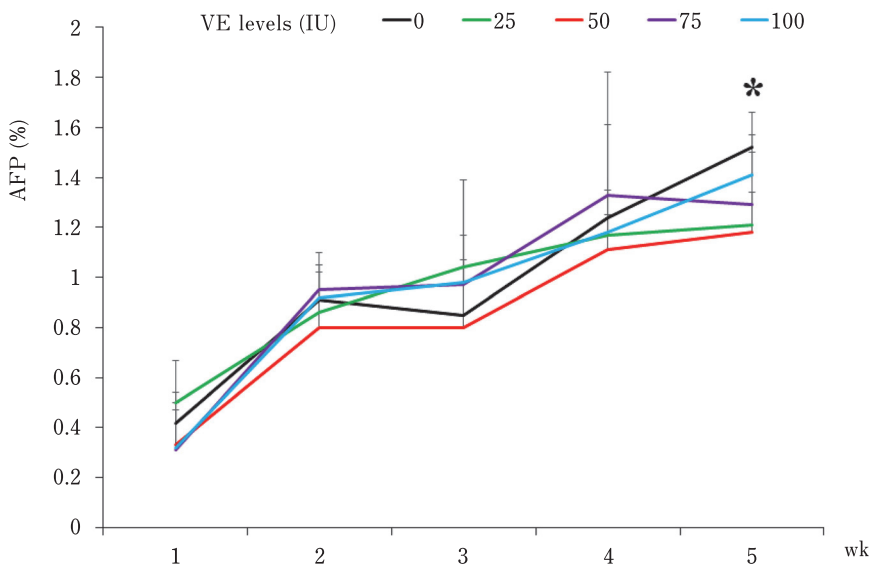

Fig. 1. **Effect of dietary vitamin E supplementation on abdominal fat deposition in broilers.**

pared to the control (P<0.05; Table 2). Abdominal fat deposition was significantly decreased after 5 weeks of 25 or 50 IU vitamin E treatment compared to the control (P<0.05; Fig. 1 and Table S1). Serum α-tocopherol levels were significantly higher in broilers that received vitamin E supplementation compared to the controls (P<0.05; Table 3).

Mapping and Counting Reads

Approximately 44 Mb clean reads for each sample, with the percentages of mapped reads ranging from 73.10% to 76.53%, were obtained after raw data quality control. There were similar clean reads and percentages of mapped reads in the FC and FT groups (Table 4). The total number of genes and transcripts in the six abdominal fat samples ranged from

Table 3. **Effect of dietary vitamin E levels on serum vitamin E contents in broilers[1]**

Items	Vitamin E levels[2]					*P*-value	Pooled SEM
	0 IU	25 IU	50 IU	75 IU	100 IU		
7 days	20.71^{d}	31.51^{a}	29.17^{ab}	25.76^{bc}	22.9^{dc}	0.00	1.43
14 days	16.68^{bc}	15.83^{bc}	17.68^{ab}	14.73^{c}	19.49^{a}	0.00	0.73
21 days	13.54^{b}	18.85^{a}	18.78^{a}	16.85^{ab}	15.22^{ab}	0.00	1.33
28 days	13.29^{c}	15.8^{ab}	17.12^{a}	14.71^{bc}	15.83^{ab}	0.00	0.62
35 days	13.35^{c}	16.78^{b}	16.16^{b}	17.04^{ab}	16.81^{a}	0.00	0.41

Note: [1] Data are means and pooled SEM (n=6); different lowercase superscripts in each column indicate significant differences.
[2] Vitmian E is DL-α-tocopheryl acetate, 1 mg DL-α-tocopheryl acetate=1 IU alpha-tocopherol.

Table 4. **Number of reads obtained and percentage of mapped reads per sample**

Sample ID[1]	Total clean reads (Mb)[2]	Clean reads ratio (%)	Total mapping ratio (%)[3]
FC1	44.08	74.78	76.51
FC2	44.64	75.73	74.13
FC3	44.92	76.20	73.49
FT1	44.90	76.17	73.75
FT2	44.54	73.52	74.60
FT3	44.61	73.63	73.10

Note: [1] FC1, FC2, FC3 and FT1, FT2, FT3 are from the abdominal fat tissues of broilers in 5th week control group (FC) and 50 IU vitamin E treatment group (FT), respectively.
[2] The clean data obtained after quality control.
[3] The clean reads mapped on the reference genome.

Table 5. **The number of genes and transcripts per sample**

Sample ID	FC1	FC2	FC3	FT1	FT2	FT3	*P*-value[1]
Total gene number	16055	16224	15945	15828	15600	15661	0.023
Known gene number	15301	15485	15217	15082	14862	14936	0.022
Novel gene number	754	739	728	746	738	725	0.701
Total transcript number	33571	33997	31314	30705	30363	30200	0.089
Known transcript number	24462	24896	22703	22182	21944	21757	0.087
Novel transcript number	9109	9101	8611	8523	8419	8443	0.096

Note: [1] *P*-value from the independent-samples t-test between the FC and FT groups.

15600~16224 and 30200~33997, respectively (Table 5 and Table S2). The total gene number and known gene number in the FC group were greater than in the FT group ($P<0.05$), but the total transcript number between the FC and FT groups were not significantly different ($P>0.05$). Sequence data that support the findings of this study have been deposited in the NCBI Gene Expression Omnibus (GEO) data repository under the primary accession code, GSE113170.

Analysis of DEGs

A total of 602 DEGs were identified between the FC and FT groups (Table S3). Compared to the FC group, there were 126 upregulated genes and 476 downregulated genes in the FT group (Fig. 2 and Table S3). Cluster analysis between biological replicates showed that the samples formed two distinct groups, FC and FT, and indicated that the DEGs were very highly reproducible in replicate samples (Fig. 3).

Functional Enrichment Analysis of the Identified DEGs

GO analysis was performed for the up- and downregulated DEGs to identify differences in gene expression in the vitamin E group that could regulate abdominal fat deposition. DEGs were found in three ontologies with 50 GO terms, including biological process, cellular component, and molecular function (Fig. 4 and Table S4). The functions of most DEGs were cellular processes, single-cell organism processes, and cell-cell adhesion.

Following pathway function analysis of the DEGs, a total of 42 signaling pathways were identified ($P<0.05$; Table S5). The DEGs were mainly enriched for metabolism, organismal systems, environmental information processing, human diseases, and cellular processes. The 11 lipid metabolism-related pathways with 83 DEGs are shown in Table 6. Among these metabolic pathways, fatty acid biosynthesis and glycerolipid metabolism are vital lipid metabolism path-

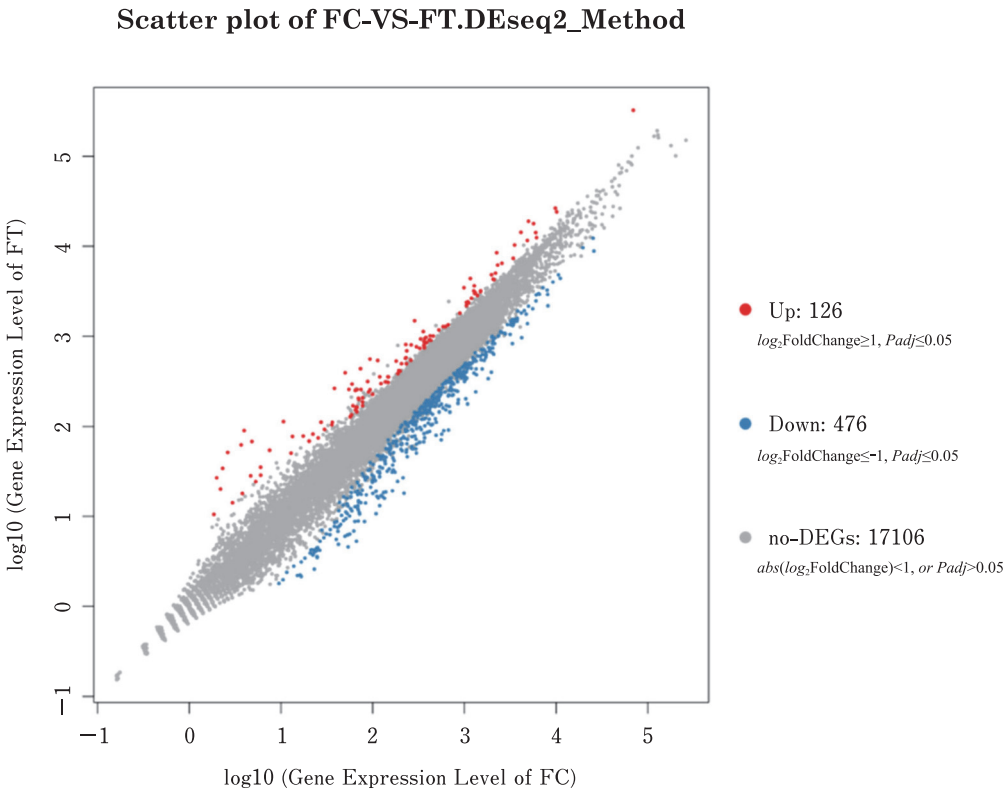

Fig. 2. **Gene expression between the FC and FT groups.** Compared with the FC group, there were 126 upregulated genes and 476 downregulated genes in the FT group.

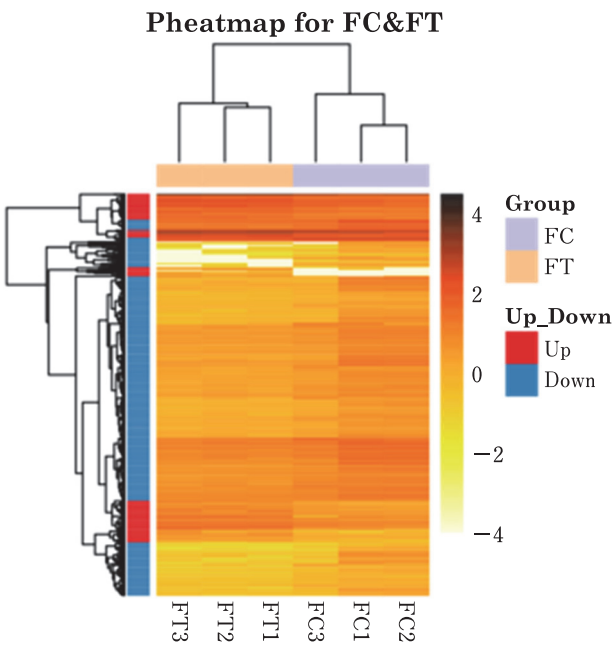

Fig. 3. **Gene Ontology (GO) analysis results for differentially expressed genes (DEGs) in the FC and FT groups.** GO analysis was performed for the up- and downregulated DEGs to identify differences in gene expression in the vitamin E group that could regulate abdominal fat deposition. The DEGs were found in three ontologies with 50 GO terms, including biological process, cellular component, and molecular function.

ways for lipogenesis; insulin resistance, and insulin signaling and have important roles in lipid metabolism. Some of the DEGs in the lipid metabolism-related pathways were important regulated-genes, such as, fatty acid synthase (*FASN*), acetyl-CoA carboxylase alpha (*ACACA*), and cAMP responsive element binding protein 5 (*CREB5*).

qPCR Validation of RNA-seq Data

Nine DEGs in the lipid metabolism-related pathways were selected to validate the RNA-seq results by qPCR. All primer sequences are listed in Table S6. All of the selected genes (*FASN*, *ACACA*, *ACSL1*, *LOC100858678*, *FOS*, *FGFR3*, *MAP3K1*, *ATP2A3*, and *LPIN1*) showed results consistent with the RNA-seq analysis (Table 7). Thus, our qPCR data confirmed the RNA-seq results, at least for the eight selected lipid metabolism genes.

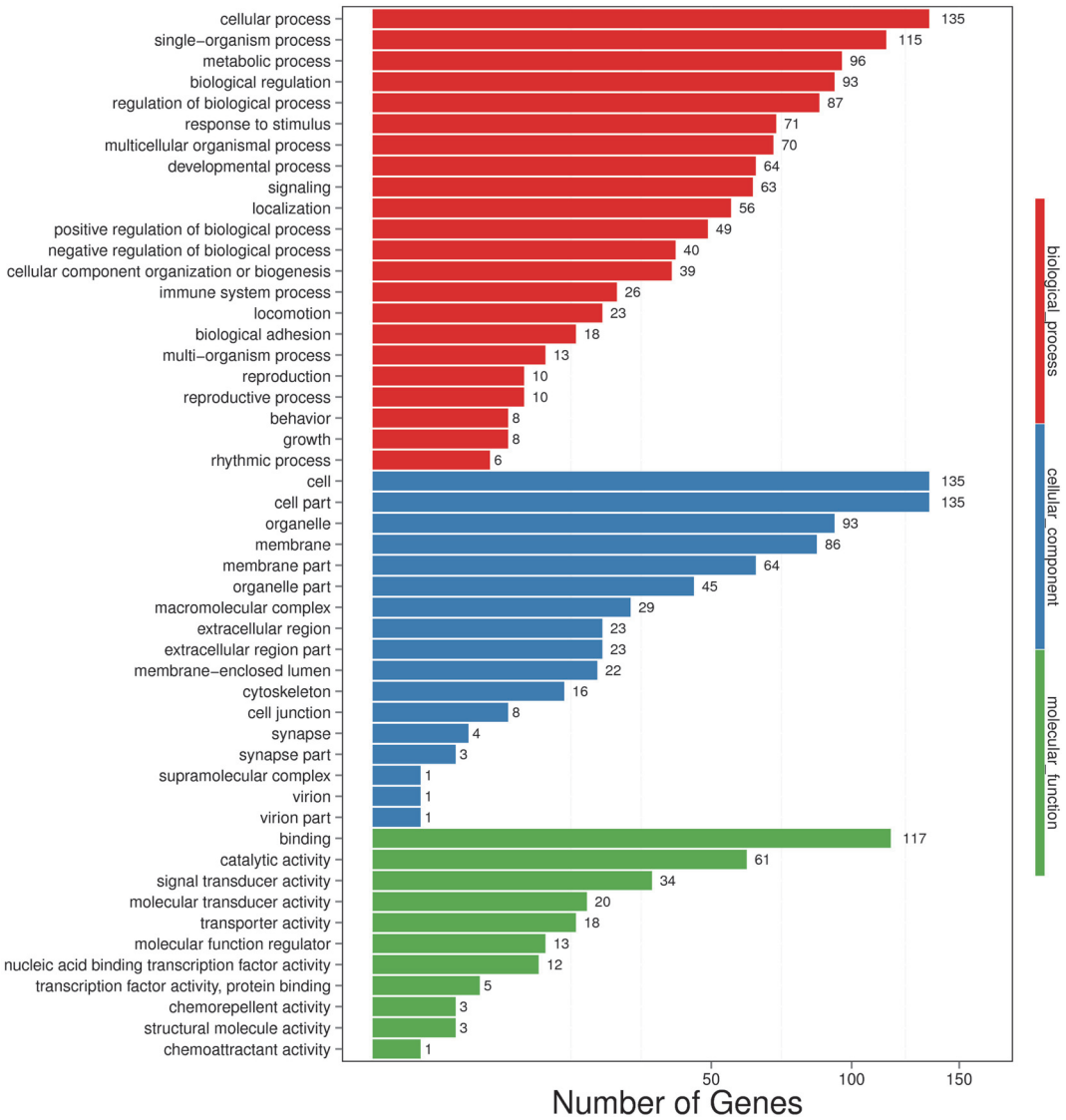

Fig. 4. **Heatmap of hierarchical clustering of differentially expressed genes (DEGs).** X axis represents the comparisons for clustering analysis; Y axis represents the DEGs. Colors indicate log2-transformed fold change (high: red; low: blue).

Discussion

Obesity causes adverse health outcomes in humans, and similar problems exist in chickens (Jin *et al*., 2017). Excessive abdominal fat accumulates in chickens, making them an ideal model organism for studying obesity-associated metabolic changes. Adipose tissue not only maintains energy balance, but also functions as a crucial endocrine organ that controls lipid metabolism (Jin *et al*., 2017). Female broilers were used in this study because they accumulate more fat than their male counterparts and they continuously deposit abdominal fat after hatching (Bai *et al*., 2015). Previously, several studies have used transcriptional analyses of abdominal fat from chickens to determine active signaling pathways and divergent gene expression for different trial objectives (Duan *et al*., 2013; Resnyk *et al*., 2015; Resnyk *et al*., 2017; Zhuo *et al*., 2015). Such studies have shown that vitamin E can modulate gene expression. Among the eight fat-soluble vitamin E compounds, α-tocopherol is the most bioactive (Sun *et al*., 2015), and DL-α-tocopherol acetate is commonly used as a source of vitamin E in the diets of broilers. The aim of this study was to use RNA-seq to reveal key metabolic pathways and genes that regulate abdominal fat deposition in broilers that were fed a diet supplemented with DL-α-tocopherol acetate in an effort to provide a global reference that could be used to prevent excessive obesity in broilers.

A meta-analysis indicated that there was no relationship

Table 6. **Lipid metabolism-related pathways represented by DEGs**

Pathways	ID	Genes[1]
Fatty acid biosynthesis	ko00061	*FASN*, *ACACA*, *ACSL1, LOC100858678*
Insulin resistance	ko04931	*FOXO1*, *PTPN11*, *PRKCB*, *MLXIP*, *TMTC4*, *OGT*, *C2CD2L*, *CREB5*, *LOC100858678*, *LOC101749060*, *LOC107048989*, *LOC107049310*
MAPK signaling pathway	ko04010	*CACNB4*, *PPP3CA*, *FGFR4*, *FOS*, *FGFR3*, *SYNGR3*, *PRKCB*, *RALGPS1*, *NALCN*, *PRDM16*, *AMHR2*, *TNIK*, *MAP3K1*, *GADD45B*, *GADD45A*, *MEF2C*, *LOC107049310*, *LOC107050212*, *LOC107050551*, *LOC107053420*, *LOC107056101*
Glycerolipid metabolism	ko00561	*PNPLA3*, *LPIN1*, *EFCAB4B*, *PPAP2A*, *LIPC, FAM126B*, *LOC107049382*
Insulin signaling pathway	ko04910	*FOXO1*, *FASN*, *ACACA*, *RALGPS1*, *ANKS1A*, *RALGAPA1*, *SORBS1*, *LOC100858678*, *PRKAR2A*, *LOC101749060*, *LOC107053420*, *LOC107056101*, *LOC107057135*
Thyroid hormone signaling pathway	ko04919	*FOXO1*, *NOTCH1*, *ANOS1*, *ITGAV*, *PRKCB*, *NCOA3*, *PLCB2*, *RALGAPA1*, *PLCE1*, *GSE1*, *PLCB1*, *KAT2B*, *LOC107053420*
Apelin signaling pathway	ko04371	*HDAC4*, *SMAD3*, *NOV*, *PIK3CG*, *PLCB2*, *PLCB1*, *MEF2D*, *MEF2C*, *JAG2*, *LOC101748756*, *LOC107048989*, *LOC107050621*, *LOC107053420*
mTOR signaling pathway	ko04150	*FZD4*, *FZD1*, *PRKCB*, *RALGPS1*, *ENOX1*, *RNF152*, *FNIP2*, *RALGAPA1*, *RICTOR*, *LOC107049310*, *LOC107053420*, *DDIT4*, *LOC107056101*
Calcium signaling pathway	ko04020	*PPP3CA*, *ATP2A3*, *PRKCB*, *NALCN*, *ATP2B4*, *PLCB2*, *HTR7*, *PLCE1*, *ADRB2*, *PLCB1*, *CYSLTR2*, *NTSR1*, *PTGER3*, *PDE1B*, *LOC101748756*, *LOC107048989*, *LOC107050621*
Estrogen signaling pathway	ko04915	*FOS*, *RALGPS1*, *FKBP5*, *PLCB2*, *PLCB1*, *CREB5*, *LOC107048989*, *LOC107053420*, *LOC107056101*
Phospholipase D signaling pathway	ko04072	*PTPN11*, *KITLG*, *IL8L2*, *RALGPS1*, *PIK3CG*, *GAB2*, *PLCB2*, *RALGAPA1*, *PPAP2A*, *PLCB1*, *CXCR1*, *LOC107053420*, *LOC107056101*

[1] New gene IDs are not shown.

Table 7. **Verification of RNA-seq data in abdominal fat from the control and vitamin E-supplemented groups**

Gene name	RNA-seq		qPCR	
	Fold change	Adjusted P-value	Fold change	P-value
FASN	−1.43	<0.001	−3.28	0.004
ACACA	−1.82	0.001	−1.82	0.001
ACSL1	−1.55	<0.001	−2.92	0.003
LOC100858678	−2.37	0.035	−4.63	<0.001
FOS	1.79	<0.001	3.42	0.010
FGFR3	−1.71	0.022	−1.01	0.003
MAP3K1	−1.27	0.012	−2.82	0.002
ATP2A3	−1.10	0.034	−1.85	0.036
LPIN1	1.20	0.032	3.80	0.019

between dietary vitamin E supplementation and growth performance in broilers (Pompeu *et al*., 2018), which was consistent with the findings of our study. Another study showed that vitamin E supplementation tended to improve growth and feed utilization during the first 0−3 weeks, while performance from 0−5 weeks was not influenced (Guo *et al*., 2001), possibly because responses to continuous vitamin E feeding are influenced by genetic stock, age, duration of feeding, and measurement criteria (Siegel *et al*., 2001). Vitamin E supplementation has consistently been shown to reduce the abdominal fat content in broilers (Li *et al*., 2009; Zaboli *et al*., 2013), which is consistent with the findings of this study. Because the effect of vitamin E needs to be accumulated, the abdominal fat deposition of broilers decreased until the fifth week.

Through RNA-seq analysis, we identified 11 lipid metabolism-related pathways that were regulated by vitamin E and themselves could regulate abdominal fat deposition in broilers. We found two particularly noteworthy lipid metabolism pathways, fatty acid biosynthesis and glycerolipid metabolism, which have been proven to have essential functions in lipogenesis and lipolysis. We identified four genes in the fatty acid biosynthesis pathway that were downregulated following vitamin E treatment: *FASN*, *ACACA*, *ACSL1,* and *LOC100858678*. *FASN* and *ACACA* are also in the insulin signaling pathway, and their protein products are

the rate-limiting steps in *de novo* fatty acid synthesis. FASN is a multifunctional enzyme, that primarily catalyzes *de novo* saturated fatty acid synthesis and regulates lipid metabolism (Wakil *et al*., 1989; Niranjan *et al*., 2016). In chickens, an association study of two single nucleotide polymorphisms (SNPs) of *FASN* and abdominal fat traits was performed using an F2-designed resource population, and the results indicated that individuals with the BB genotype had a higher fat deposition capacity than other SNPs in the Hae III site; when heterozygotes of the two genotypes were tested, individuals with a BC/GG genotype had the highest fat deposition capacity (Ouyang *et al*., 2007). Through comparative genomic approaches, important candidate lipid metabolism-related genes were identified, including *FASN* (Bakhtiarizadeh *et al*., 2013). *ACACA* encodes acetyl-coenzyme A carboxylase, which catalyzes the carboxylation of acetyl-CoA to form malonyl-CoA and is the rate limiting and committed step of *de novo* fatty acid biosynthesis (Calvo *et al*., 2000). Using a combination of transcriptomics and metabolomics, a previous study found that *ACACA* was downregulated in the adipose tissue of chickens during fasting compared to fed controls (Ji *et al*., 2012). A transcriptional analysis of abdominal fat from genetically fat and lean chickens also found that *ACACA* was downregulated in lean chickens (Resnyk *et al*., 2013). The epistatic effect between *ACACA* and *FABP2* affected the phenotypic variation of abdominal fat content in broilers (Hu *et al*., 2010). Acyl-CoA synthetase long-chain family member 1 (*ACSL1*) encodes long-chain fatty acid-CoA ligase 1, which was downregulated in this study. Another previous study showed that microRNA gga-miR19b-3p contributed to the increased accumulation of abdominal fat in Beijing-You chickens by downregulating *ACSL1* (Huang *et al*., 2015). These inconsistent results may be due to the different breeds of chickens used in the two studies. Seven glycerolipid metabolism-associated genes, including *PNPLA3*, *LPIN1*, *EFCAB4B*, *PPAP2A*, *LIPC*, *FAM126B,* and *LOC107049382* were identified in this study. This pathway was also identified in abdominal fat tissue between fast and slow growing chickens (Claire *et al*., 2014). Based on this and previous studies, the fatty acid biosynthesis pathway could be a key vitamin E-regulated pathway that controls abdominal fat metabolism in broilers; and *FASN* and *ACACA* may be key vitamin E-regulated genes that also affect this process.

Insulin and insulin resistance are well-known lipid metabolism-related pathways that play important roles in lipid metabolism and obesity resistance (Saltiel *et al*., 2001; Kahn *et al*., 2006). A previous study that analyzed lncRNA and mRNA expression by RNA-seq showed that mTOR and MAPK signaling were active in chicken abdominal preadipocytes at different stages of differentiation (Zhang *et al*., 2017). Both signaling pathways play essential roles in preadipocyte differentiation and were also identified in this study. Other signaling pathways, including apelin signaling, thyroid hormone, calcium, estrogen, and phospholipase D also play important roles in lipid metabolism, and require further study with regards to their effects on abdominal fat metabolism in broilers.

In conclusion, we found that vitamin E inhibits abdominal fat deposition in broilers through the fatty acid biosynthesis pathway and we identified key genes that regulate lipid metabolism. It is worth mentioning that the genes encoding the rate-limiting steps of *de novo* fatty acid synthesis, *FASN* and *ACACA*, showed decreased expression in abdominal fat tissue following vitamin E supplementation. In practice, dietary vitamin E supplementation is insufficient for boiler chickens, and may lead to more abdominal fat accumulation through regulated genes, especially *FASN* and *ACACA*. Herein, we identified the pathways and genes regulated by vitamin E, thereby providing new insights into vitamin E regulation of abdominal fat deposition in broilers.

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Conflicts of Interest

The authors declare no conflict of interest.

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