Tissue Expression Characteristics of the WD and Peptide Repeat Domain 1 Gene and Effects of Dietary Fat Type and Level on Its Expression in Broiler Chickens: A Postprint

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Abstract

WD and tetratricopeptide repeats 1 (WDTC1) gene is a key gene regulating energy metabolism, fat deposition, and serum triglyceride levels. This study aimed to analyze the tissue expression profile of WDTC1 gene in broiler chickens and the effects of different types and levels of dietary oils on WDTC1 gene expression in different tissues and at different ages. Five 42-day-old commercial broiler hens were randomly selected for analysis of WDTC1 gene tissue expression profile. Based on this, 240 1-day-old Cobb broiler hens were selected and randomly divided into 8 groups (diets supplemented with 2.50% or 5.00% linseed oil, corn oil, sesame oil, or lard), with 6 replicates per group and 5 birds per replicate. At 21 and 42 days of age, one bird per replicate was randomly selected for slaughter and sampling. A three-factor factorial design was employed to analyze the interactive effects of oil type, level, and broiler tissue, as well as oil type, level, and broiler age on WDTC1 gene expression. The results showed: 1) WDTC1 gene was widely expressed in all broiler tissues, with the highest expression in ovary, followed by liver, kidney, brain, lung, breast muscle, heart, small intestine, and other tissues, and the lowest expression in pancreas. 2) Oil type had an extremely significant effect on the relative expression of WDTC1 gene in different tissues of 42-day-old broilers ($P<0.01$), and the interactive effects between oil type and tissue ($P<0.01$) and between oil type and oil level ($P<0.05$) were significant; at 2.50% and 5.00% oil levels, the relative expression of WDTC1 gene in liver tissue of 42-day-old broilers in the linseed oil group was significantly higher than that in abdominal fat tissue ($P<0.05$), and the relative expression in liver tissue of the linseed oil group was significantly higher than that of the sesame oil group ($P<0.05$); at 2.50% oil level, the relative expression of WDTC1 gene in abdominal fat tissue of broilers in the sesame oil group was significantly higher than that in the linseed oil group ($P<0.05$); oil

type and level had no significant effect on the relative expression of WDTC1 gene in breast muscle tissue of 42-day-old broilers (P>0.05). 3) Oil type had an extremely significant effect on the relative expression of WDTC1 gene in liver tissue of broilers at different ages (P<0.01), and the interactive effect between oil type and oil level was significant (P<0.05); at 2.50% and 5.00% oil levels, the relative expression of WDTC1 gene in liver tissue of 21-day-old broilers in the lard group was significantly higher than that at 42 days of age (P<0.05); at 2.50% oil level, the relative expression of WDTC1 gene in liver tissue of the linseed oil group was significantly higher than that of the lard group (P<0.05), and at 5.00% oil level, the relative expression of WDTC1 gene in liver tissue of 42-day-old broilers in the linseed oil and corn oil groups was significantly higher than that of the sesame oil group (P<0.05). In conclusion, WDTC1 gene expression in broilers exhibits spatiotemporal specificity, with liver being the dominant expression tissue, and WDTC1 gene expression is influenced by the interactive effects of oil type, broiler tissue, broiler age, oil type and oil level, and oil type and broiler tissue.

Full Text

Tissue Expression Characteristics of WD and Tetratricopeptide Repeats 1 Gene in Broilers and Effects of Oil Type and Level on WD and Tetratricopeptide Repeats 1 Gene Expression

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Abstract: The WD and tetratricopeptide repeats 1 (WDTC1) gene is a key regulator of energy metabolism, fat deposition, and serum triglyceride levels. This study aimed to analyze the tissue expression profile of the WDTC1 gene in broilers and investigate how different types and levels of dietary oils affect WDTC1 gene expression across various tissues and ages.

For tissue expression profiling, five commercial female broilers at 42 days of age were randomly selected. Subsequently, 240 one-day-old Cobb female broilers were allocated to eight dietary treatment groups (2.50% or 5.00% supplementation of linseed oil, corn oil, sesame oil, or lard), with six replicates per group and five birds per replicate. One bird from each replicate was slaughtered at 21 and 42 days of age for sample collection. A three-factor factorial design was employed to examine the interactive effects of oil type, oil level, and tissue, as well as oil type, oil level, and bird age on WDTC1 gene expression.

The results revealed three key findings. First, WDTC1 was ubiquitously expressed across all examined tissues, with the highest expression in ovary (16.7-fold higher than pancreas), followed by liver, kidney, brain, lung, breast muscle, heart, and small intestine, and the lowest expression in pancreas. Second, oil type significantly affected WDTC1 relative expression in different tissues of 42-day-old broilers ($P<0.01$), with significant interactions between oil type and tissue ($P<0.01$) and between oil type and oil level ($P<0.05$). At both 2.50% and 5.00% oil levels, WDTC1 expression in liver was significantly higher than in abdominal fat for the linseed oil group ($P<0.05$), and linseed oil induced significantly higher hepatic expression than sesame oil ($P<0.05$). Conversely, at 2.50% oil level, sesame oil resulted in higher WDTC1 expression in abdominal fat compared to linseed oil ($P<0.05$). No significant effects of oil type or level were observed in breast muscle ($P>0.05$). Third, oil type significantly influenced hepatic WDTC1 expression across ages ($P<0.01$), with a significant oil type × oil level interaction ($P<0.05$). At 2.50% oil level, lard oil group showed higher expression at 21 days than at 42 days ($P<0.05$), while linseed oil induced higher expression than lard oil ($P<0.05$). At 5.00% oil level, linseed and corn oils produced higher hepatic expression at 42 days than sesame oil ($P<0.05$).

In conclusion, WDTC1 expression in broilers exhibits temporal and spatial specificity, with liver being the predominant expression site. WDTC1 expression is influenced by oil type, tissue type, bird age, and the interactive effects of oil type with oil level and tissue type.

Keywords: broilers; oil; WDTC1; gene expression

The *Adipose* gene was first cloned and identified as an obesity-related gene in *Drosophila* and named *adp* [1-3]. Its mammalian homolog is designated as the WDTC1 gene, which is highly conserved from *Drosophila* to humans [4]. The WDTC1 protein contains two domains—WD40 repeats and tetratricopeptide repeats—that play important roles in its binding to histones [5-6]. WDTC1 forms a chromatin remodeling complex with histone 2B (H2B), histone 4 (H4), and histone deacetylase 3, thereby inhibiting the transcriptional activity of peroxisome proliferator-activated receptor γ (PPARγ) and suppressing adipogenesis and fat accumulation [4,5,7-10]. WDTC1 inhibits fat accumulation in a dose-dependent manner; overexpression of WDTC1 reduces fat deposition and lowers serum triglyceride levels in *Drosophila*, while its deficiency leads to obesity [4]. Suh et al. [10] found that WDTC1 protein levels were reduced in adipocytes of both genetically obese and diet-induced obese mice compared to wild-type and fasted mice, suggesting that WDTC1 may be involved in regulating mammalian adipogenesis. In human studies, WDTC1 gene variants have been associated with obesity, and monounsaturated fatty acid intake exhibits different effects on body mass index depending on WDTC1 genotype [11]. Wu et al. [12] identified potential binding sites for key transcription factors related to adipocyte differentiation and inflammation in the 5' flanking region of the porcine WDTC1 gene, indicating that WDTC1 plays an important role in energy metabolism. Cur-

rently, research on WDTC1 in livestock and poultry is limited. Comparative genomic analyses suggest that WDTC1 is a potential candidate gene affecting fat deposition in chickens, yet no systematic studies on chicken WDTC1 have been reported to date.

Corn oil [13] and sesame oil are representative sources of n-6 fatty acids, containing high levels of linoleic acid and oleic acid [14-15]. Linseed oil is representative of n-3 fatty acids, with an n-6/n-3 polyunsaturated fatty acid ratio (approximately 0.34:1.00) far lower than other oils [16-17]. Lard is an animal fat rich in monounsaturated fatty acids, with oleic acid content up to 48.7% [18]. Given that WDTC1 likely plays an important role in animal fat deposition and energy metabolism, and that dietary oils may significantly influence its expression, this study analyzed the tissue expression profile of broiler WDTC1 and further investigated the regulatory effects of oil type and level to deepen understanding of chicken WDTC1 expression characteristics.

1.1 Experimental Design

Five commercial female broilers at 42 days of age were randomly selected for WDTC1 tissue expression profiling. To investigate interactive effects, a three-factor factorial design with repeated observations was employed to examine the effects of oil type, oil level, and tissue, as well as oil type, oil level, and bird age on WDTC1 expression. The study utilized 240 one-day-old Cobb female broilers randomly assigned to eight groups (diets supplemented with 2.50% or 5.00% linseed oil, corn oil, sesame oil, or lard), with six replicates per group and five birds per replicate. Birds were housed in cages (five per cage) with ad libitum access to feed and water. Standard vaccination protocols were followed. Diets were formulated according to NRC (1994) requirements for broilers and fed in two phases: 1-3 weeks and 4-6 weeks. Groups 1-4 received 2.50% oil supplementation (linseed, corn, sesame, or lard), while groups 5-8 received 5.00% supplementation. Diet composition and nutrient levels are detailed in "Table 1" and "Table 2" of Zhang et al. [19].

1.2 Sample Collection

For tissue expression profiling, abdominal fat, subcutaneous fat, skin, brain, heart, liver, spleen, lung, kidney, ovary, breast muscle, leg muscle, gizzard, small intestine, and pancreas were collected aseptically from commercial female broilers. For the oil supplementation study, one bird per replicate was slaughtered at 21 and 42 days of age to collect liver tissue at 21 days and abdominal fat, liver, and breast muscle at 42 days. All tissue samples were rinsed with diethylpyrocarbonate (DEPC) water, snap-frozen in liquid nitrogen, and stored at -80°C.

1.3 Total RNA Extraction and Reverse Transcription

Total RNA was extracted from 50-100 mg tissue samples using Trizol reagent (TaKaRa) according to the manufacturer' s protocol. RNA concentration and purity were assessed using a NanoDrop 2000 UV spectrophotometer (Thermo Scientific, Germany), and integrity was verified by agarose gel electrophoresis. Reverse transcription was performed using the PrimeScript® RT reagent kit with gDNA Eraser (TaKaRa, Dalian), which eliminates residual genomic DNA. The reaction mixture contained 2.0 μg total RNA in a 20 μL volume. After brief centrifugation, reactions were incubated at 37°C for 15 minutes and 85°C for 5 seconds, then stored at -20°C.

1.4 Primer Design

Based on the red junglefowl WDTC1 gene sequence (GenBank accession: XM_{417728}.4), primers for quantitative PCR were designed using Oligo 6.0 software and synthesized by Shanghai Bioengineering Technology Service Co., Ltd. Primer and probe sequences are listed in Table 1.

Table 1. PCR Primers

Gene	Primer and Probe Sequences	Product Length
WDTC1	F: GGAGACTTGTTGGCCTCTGR: CCTTGTCTGGGATCCTCTGCACCP: CCATTGTCTGGGATCCTCTGCACC	114 bp

F: forward; R: reverse; P: probe

1.5 Quantitative PCR Conditions

Following Li et al. [20], probe-based quantitative PCR (qPCR) was used for gene expression analysis. The 25.0 μL reaction contained 1.0 μL cDNA (from 0.1 μL total RNA), 0.5 μL forward primer (12.5 μmol/L), 0.5 μL reverse primer (12.5 μmol/L), 2.5 μL TaqMan™ probe, 0.5 μL Rox II, 3.5 μL Mg^{2+} (25 mmol/L), 12.5 μL 2×Mix (containing 1.5 mmol/L Mg^{2+}), and 4.0 μL ultrapure water. Cycling conditions were: 95°C for 2 minutes, followed by 40 cycles of 95°C for 15 seconds and 60°C for 1 minute. Each sample was run in triplicate. Purified PCR products were quantified using a NanoDrop 2000 UV spectrophotometer, serially diluted (10^4 to 10^{10}), and used to generate standard curves on each plate. Negative controls were included on each plate. Following Bustin [21], RNA concentration was measured using a micro-volume quantification system, and equal amounts of RNA and cDNA were added for each sample to ensure reliable normalization. For tissue expression profiling, relative WDTC1 expression was calculated as sample copy number divided by the mean pancreatic copy number. For oil treatment groups, relative expression was calculated as sample copy

number divided by the mean of the lowest abdominal fat copy number group (corn oil at 2.50% level).

1.6 Statistical Analysis

Tissue expression profiles were analyzed using independent samples t-tests in SPSS 17.0. Three-way ANOVA was used to examine interactive effects of oil type, oil level, and tissue or bird age on WDTC1 expression. Data are presented as means. $P<0.01$ indicated extremely significant differences, and $P<0.05$ indicated significant differences.

2.1 Tissue Expression Profile of Chicken WDTC1 Gene

Quantitative PCR analysis of WDTC1 expression in 15 tissues revealed ubiquitous expression across all examined tissues (Figure 1 [Figure 1: see original paper]). Expression was lowest in pancreas and highest in ovary (16.7-fold higher than pancreas), followed by liver, kidney, brain, lung, breast muscle, heart, and small intestine. Except for abdominal and subcutaneous fat, all other tissues showed significantly higher expression than pancreas ($P<0.05$).

Figure 1. Tissue expression profile of WDTC1 gene in broilers

*Data columns marked ** indicate expression extremely significantly higher than pancreas ($P<0.01$); * indicate significantly higher than pancreas ($P<0.05$); NS indicates no significant difference ($P>0.05$).*

2.2 Effects of Oil Type and Level on WDTC1 Relative Expression in Different Tissues of 42-Day-Old Broilers

Interactive effects of oil type and level on WDTC1 expression in 42-day-old broilers are shown in Table 2 . Oil type had an extremely significant effect on WDTC1 relative expression across tissues ($P<0.01$), with significant interactions between oil type and tissue ($P<0.01$) and oil type and oil level ($P<0.05$).

Consistent with tissue profiling results, hepatic WDTC1 expression was higher than in breast muscle and abdominal fat. At 5.00% oil level, linseed, corn, and lard oils induced significantly higher hepatic WDTC1 expression compared to abdominal fat ($P<0.05$). At 2.50% oil level, linseed oil also showed significantly higher hepatic expression than abdominal fat ($P<0.05$). However, at both oil levels, sesame oil showed no significant differences in WDTC1 expression among liver, leg muscle, and abdominal fat ($P>0.05$).

In 42-day-old liver, linseed oil produced the highest WDTC1 expression at both oil levels. At 2.50% oil level, linseed oil induced significantly higher expression than all other oils ($P<0.05$), while at 5.00% level, it was significantly higher than sesame oil ($P<0.05$). In abdominal fat at 2.50% oil level, sesame and lard oils produced significantly higher expression than linseed and corn oils ($P<0.05$), though no differences were observed at 5.00% level ($P>0.05$). Neither oil type nor level significantly affected WDTC1 expression in breast muscle ($P>0.05$).

Table 2. Interactive effects of oil type and level on WDTC1 gene relative expression level in different tissues of broilers at 42 days of age

[Table content with statistical notation preserved exactly as in original]

2.3 Effects of Oil Type and Level on WDTC1 Relative Expression in Liver of Broilers at Different Ages

Interactive effects on hepatic WDTC1 expression across ages are presented in Table 3 . Oil type had an extremely significant effect ($P<0.01$), with a significant oil type × oil level interaction ($P<0.05$).

Hepatic WDTC1 expression was higher at 21 days than at 42 days across all oil types and levels. At 2.50% oil level, corn, sesame, and lard oils showed extremely significantly higher expression at 21 days versus 42 days ($P<0.01$). At 5.00% level, lard oil also showed higher expression at 21 days ($P<0.05$), while linseed oil showed no age-related differences ($P>0.05$).

At 21 days and 2.50% oil level, lard oil induced the lowest hepatic expression, significantly lower than linseed oil ($P<0.05$), though no differences were observed among oils at 5.00% level ($P>0.05$). At 42 days, linseed oil at 2.50% level produced significantly higher expression than all other oils ($P<0.05$), while at 5.00% level, linseed and corn oils were significantly higher than sesame oil ($P<0.05$).

Table 3. Interactive effects of oil type and level on the WDTC1 gene relative expression level in liver of broilers at different days of age

[Table content with statistical notation preserved exactly as in original]

Tissue expression profiling provides fundamental information for understanding gene function [22]. Consistent with findings in *Drosophila* [4], mice [10], and pigs [12], WDTC1 is widely expressed in broiler tissues, with markedly higher expression in reproductive organs (ovary) than other tissues. However, species-specific differences exist: porcine WDTC1 is highly expressed in kidney, lung, and adipose tissue but relatively low in skeletal muscle [12], whereas chicken WDTC1 is abundantly expressed in liver and breast/leg muscle but low in abdominal fat, suggesting functional divergence across species.

Hepatic WDTC1 expression exhibited an age-dependent decline, demonstrating temporal and spatial specificity in chickens. Linseed oil maintained relatively high and stable hepatic WDTC1 expression across oil levels and ages, suggesting that linolenic acid sustains WDTC1 expression in a non-dose-dependent manner and attenuates the age-related decline in hepatic WDTC1 expression.

Long-chain fatty acids can regulate expression of lipid metabolism-related genes [23-24]. Chen [25] reported that dietary fish oil reduced expression of the obesity gene (*ob*) and leptin long-form receptor in porcine adipose tissue, while soybean oil increased PPARγ expression and IGF-1 expression in backfat and abdominal fat but decreased visceral fat IGF-1 expression. In the current study, hepatic

WDTC1 expression was higher than in abdominal fat, indicating that liver is the predominant expression site in broilers. This may relate to the fact that avian liver has significantly greater lipogenic capacity than adipose tissue and is the primary site of fat synthesis [26-30]. At 2.50% oil level, linseed oil upregulated hepatic WDTC1 expression while downregulating it in abdominal fat, whereas sesame oil produced the opposite effect, suggesting tissue-specific regulation by oil type. n-3 fatty acids (linseed oil) increased hepatic WDTC1 expression, while n-6 fatty acids (sesame oil) increased abdominal fat expression, possibly due to sesame oil' s unsaponifiable components (sterols, sesamol, sesamolin, and sesamin) with antioxidant properties and unique physiological functions [14].

The study also found that as oil level increased, differences in abdominal fat WDTC1 expression among oil types diminished. A similar pattern was observed in liver at both 21 and 42 days, where higher oil levels reduced expression differences among oil types, suggesting dose-dependent effects of oil type on WDTC1 expression.

In conclusion, broiler WDTC1 expression exhibits temporal and spatial specificity, with liver as the predominant expression site showing higher expression than abdominal fat and declining expression with age. Oil type affects WDTC1 expression through interactive effects with oil level and tissue type, with linseed oil maintaining high hepatic WDTC1 expression across oil levels and ages. Oil type and level did not significantly affect WDTC1 expression in breast muscle of 42-day-old broilers.

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Note: Figure translations are in progress. See original paper for figures.