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Correlation Analysis of Relative Expression of Apob, Adfp and Fatp1 with Lipid Metabolism in Daweishan Mini Chickens

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ABSTRACT

Quantitative RT-PCR was applied to measure the relative expression levels of the adipose differentiation-related protein (ADFP) gene, fatty acid transport protein 1 (FATP1) gene and apolipoprotein B (ApoB) gene in subcutaneous fat, abdominal fat, liver and muscle at five growth stages (28, 49, 70, 91 and 112 d) to determine the effect of the expression ofthese genes on fat deposition in Daweishan Mini chickens. The relative expression of ADFP gene mRNA in the abdominal fat andthe liver was significantly different between 49 d and 70 d ($p<0.05$). The relative ApoB gene expression on 91d was higher in the liver, followed by muscles,subcutaneous fat, and abdominal fat, and was significantly higher in the liver than in the other three tissues. FATP1 gene expression in the liver presented a significant positive correlation with subcutaneous fat thickness ($p<0.05$). The results of this study suggest that the three genes may control the fat development in Daweishan Mini chicken.

INTRODUCTION

Broilers are considered to have high quality when they are fleshy, and proper meat fat content can contribute for this trait. However, excessive carcass fat may affect meat flavor, and result in waste and pollution. Therefore, in poultry breeding, fat carcass content is an important indicator of chicken meat quality. Studies on the genetic mechanisms of fat synthesis and regulation may contribute for the genetic improvement and production of high-quality chickens.

Daweishan Mini chicken (Figure 1) is a rare native breed of Pingbian County, Yunnan Province, China. It is a local variety used for both meat and egg production, as well as ornamental purposes. It is called "Fragrant Chicken", it is adaptable and resistant to diseases, and hasfleshy body, well-developed breast and legs, thin bones, and high meat yield. In addition, its meat contains low fat and cholesterol level, has meaty flavor, and it is tender, when reared under extensive management conditions.If its nutritional requirements are met, it has high growth rate and matures early.

Figure 1 – Daweishan Mini chicken

Fat deposition involves numerous signal transduction and metabolic pathways, including many functional genes and transcriptional regulatory factors, which playimportant roles in fat synthesis, transport, storage, and decomposition. Adipose differentiation-related protein (ADFP) is a phospholipid lipid droplet protein that promotes the uptake of long-chain fatty acids, stimulates lipid accumulation and the formation of lipid droplets, maintainsthe reserves of triglycerides, and it is involved in the synthesis, transfer, and metabolism of lipids(Imamura *et al*., 2002; Listenberger *et al*., 2007). Fatty acid transport protein 1 (FATP1) is a member of the fatty acid transport protein family (FATPs). It is an integrated transmembrane protein involved in the transmembrane transport of fatty acids and in fatty acid metabolism, and the gene that expresses FATP1 is one of the key genes affecting body fat content of the body (Binnert *et al*., 2000). Because FATP1 can indirectly influence lipid profile and deposition by regulating fatty acid uptake and metabolism in the skeletal muscle, the determination of its expression may be important for the genetic improvement of meat traits(Poh *et al*., 2004). Apolipoprotein B (ApoB) is one of the proteins with highest molecular weight and one of the most hydrophobic of the apolipoprotein family. It is the ligand and the main structural protein of triglyceride-rich low-density lipoprotein (LDL) receptor, which plays an important role in the transport and metabolism of lipids and energy (Glickman *et al*., 1986; Schumaker *et al*., 1994). ApoB is closely related to the body weight, abdominal fat and other growthrelated traits.

The objectives of this study were to measure the expression levels of three candidate genes (ApoB, ADFP, FATP1) in different tissues of Daweishan Mini chickens at different developmental stages,as well as to analyze the correlation between the expression of these genes and carcass fat traits to determine their role in carcass fatdeposition. The results may aid the screening of molecular markers, and thus,molecular marker assisted breeding, contributing for the production and conservation of Daweishan Mini chickens.

MATERIALS AND METHODS

Animals and sample preparation

Daweishan Mini chickens were obtained from a Daweishan Mini chickens farm located in Pingbian County, Honghe city, Yunnan province, China, andsacrificed at five growth stages (28, 49, 70, 91 and 112d). Four individuals per stage were evaluated. Blood was collected from the jugular vein, and the following tissues were thensampled: subcutaneous fat from the back midline and the front of the tail sebaceous glands, and abdominal fat, liver, muscle tissue from the abdominal cavity.Tissue samples were immediately placed in a centrifuge tube containinga tissue storage solution RNA Save (AB, USA). Live weight at slaughter, heavy comb and subcutaneous fat thickness (at the back midlineand anterior to the uropygeal gland) after slaughter were determined.

Primer design

In this study, the Primer Premier 5.0 software was used to design four pairs of primers for the ADRP (NM_001031420.1), ApoB (NM_001044633.1), FATP1 (NM_001039602.2) and β-actin (NM_205518.1) gene mRNA region (Table 1).

Table 1 – Primer sequence

Primer name	5′ to 3′	Number of bases (bp)
ADFP-F	GTA TTT CTT TGC GGG CTC T	19
ADFP -R	GAT GGT TAT CCT TCG TGG T	19
ApoB -F	GCA GCC TAT GGA ACA GA	17
ApoB -R	TAG TGG AAC GCA GAG CA	17
FATP1-F	CCT TGT TGA CTC CGG GTAT	19
FATP1-R	TGG GCT CTG GTG TTC TTC	18
β-actin-F	CAG TGC TGT CTG GTG GTA	18
β-actin-R	TCT GCT GGA AGG TGG A	16

Extraction and reverse transcription of genomic RNA

Total RNA of Daweishan Mini chicken was directly extracted from tissues by TRNzol-A+ Reagent (Tian'gen, Beijing, China). The Ultra Micro Nucleic Acid Protein Analyzer (AJ, Germany) was used to determine RNA concentration (400 ng/μL or so) and purity (OD260/OD280=1.8 ~ 2.0). The reagents and conditions of FastQuant cDNA first strand synthesis kit (Tian'gen, Beijing, China) were used to synthesize cDNA by reverse transcription.

Quantitative PCR system and conditions

The kit SuperReal PreMix Plus (SYBR Green; Tian'gen, Beijing, China) was used to optimize real-time PCR reaction conditions, and to determine the reaction system (20 μL): 2 × SuperReal PreMix Plus 10 μL, 0.6 μL (10μmol/L) of each upstream and downstream primers, cDNA 1 μL (about 100 ng), 50 × ROX Reference DyeΔ 0.4 μL, and ddH2O 7.4 μL. Quantitative PCR amplification was carried out in triplicate for each sample. Amplification parameters were 40 cycles of denaturation at 94 °C for 3s, annealing at 60 °C for 30 s, extension at 72 °C for 20s.

Statistical analysis

Excell2007 was used to count Ct(cycle threshold) values of each sample. Relative quantitative analysis was performed by $2^{-\Delta\Delta Ct}$ method. SPSS.17 software was used to accomplish the multiple comparisons among target genes relative expression (Duncan's test), and to analyze the correlation between target genes relative expression and developmental changes of the evaluated traits (Person method).

RESULTS AND ANALYSIS

Melting curve of target gene and reference gene

Total RNA concentration of all samples was about 400ng/μL, and the OD260/OD280 was between 1.8 and 2.0. Therefore all thesamples met the experimental requirements. The melting curve (Fig 2) showed that the amplification product of all genes had the same peak, high reproducibility, and that there were no primer-dimer and non-specific peak. The results revealed that the fluorescence signal of the amplification process was the specific amplification product.

Correlation analysis between ADFP gene expression and the fat traits

The average Ct value of subcutaneous fat at 28d was used as control to compute the relative quantity of the *ADFP* gene in the four evaluated tissues at five stages. The results showed that the relative expression of the *ADFP* gene was not significantly different among tissues at five growth stages (28, 49, 70, 91 and 112 d) ($p>0.05$). The relative expression of *ADFP* gene in subcutaneous fat and muscle was not differentamong time points ($p>0.05$), but was significantly different between 49 d and 70 d ($p<0.05$) (Table 2).

The correlation between the relative expression of *ADFP* gene and the fat traits was analyzed by the Pearson's method. The results showed that relative expression levels of *ADFP* gene in subcutaneous fat, abdominal fat, liver, muscle were not significant correlated with fat traits (subcutaneous fat thickness, comb weight and live weight) ($p>0.05$) (Table 3).

Correlation analysis between FATP1 gene expression and fat traits

The average Ct value of subcutaneous fat at 28d was used as control to compute the relative quantity of the *FATP1* gene in the four evaluated tissues at five stages. The results showed that the relative expression of *FATP1* gene was not significantly different among tissues at five growth stages (28, 49, 70, 91 and 112d) ($p>0.05$),and was not influenced by chicken age ($p>0.05$) (Table 4).

The Pearson method was used to analyze the correlation between *FATP1* gene expression and the

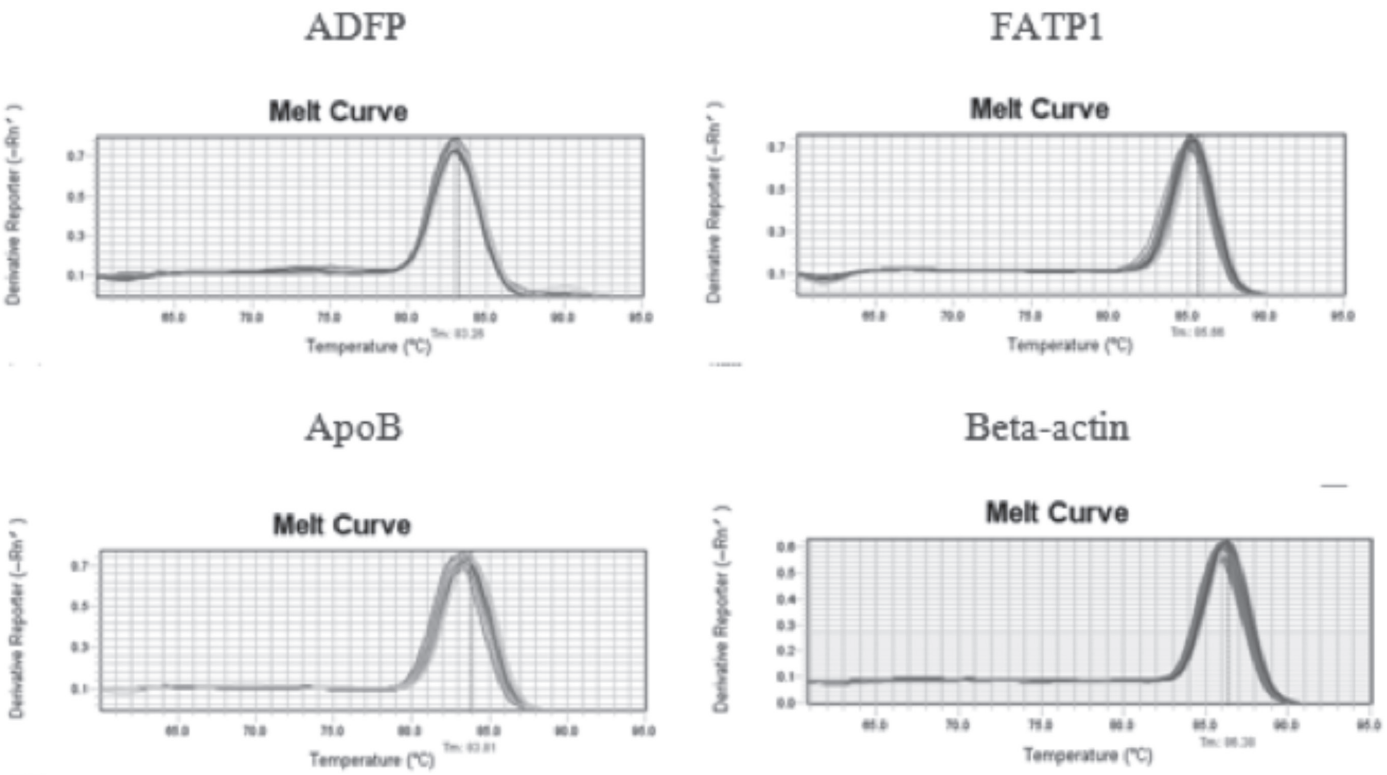

Figure 2 – Melting curve of quantitative PCR

Table 2 – Relative expression of the ADFP gene in Daweishan Mini chicken

Tissue	Days/d				
	28	49	70	91	112
Sebum	1.000	0.578±0.481	0.808±0.087	0.767±0.123	1.026±0.303
Fat	0.887±0.173	0.237±0.091^{A}	2.491±0.462^{B}	0.953±0.239	1.000±0.298
Liver	0.607±0.294	0.167±0.062^{A}	2.332±0.350^{B}	1.445±0.328	1.528±0.310
Muscle	2.579±0.447	1.306±0.650	2.550±0.612	0.950±0.136	1.973±0.266

^{A,B} Values followed by different uppercase superscripts in the same row are significantly different ($p<0.05$).

Table 3 – Correlation analysis of relative expression of ADFP gene in different tissues and fat traits

Traits		Sebum	Fat	Liver	Muscle
Live weight	R	0.316	0.114	0.533	-0.333
	P	0.604	0.856	0.355	0.584
Sebum thickness	R	0.510	0.178	0.431	0.167
	P	0.380	0.774	0.469	0.788
Comb weight	R	0.344	0.159	0.559	-0.246
	P	0.571	0.799	0.327	0.690

Note: R = correlation coefficient, P = probability.

Table 4 – Relative expression of the FATP1 gene in Daweishan Mini chicken

Tissue	Days/d				
	28	49	70	91	112
Sebum	1.000	0.719±0.201	1.201±0.523	1.375±0.426	0.818±0.126
Fat	0.646±0.117	0.962±0.240	0.924±0.166	0.671±0.199	0.501±0.191
Liver	1.021±0.115	1.595±0.265	1.743±0.212	1.156±0.093	2.544±0.793
Muscle	0.651±0.104	1.132±0.278	1.086±0.239	1.098±0.327	1.144±0.335

fat traits. The results showed that relative expression of *FATP1* gene in the liver was significantly and positively correlated with subcutaneous fat thickness ($p<0.05$), but the relative expression of *FATP1* gene in the other three tissues had no significant correlation with fat traits ($p>0.05$) (Table 5).

Correlation analysis between ApoB gene expression and fat traits

The average Ct value of subcutaneous fat at 28 d was used as control to compute the relative quantity of the *ApoB* gene in four evaluated tissues at five stages. The relative expression of *ApoB* gene was not significantly different among tissues at four growth stages (28, 49, 70 and 112d) ($p>0.05$). However, the relative expression of *ApoB* gene at 91d was significantly higher in the liver compared with the muscle, subcutaneous fat,and abdominal fat. The relative expression of ApoB gene was significantly higher on d 91 compared with 21d and 112d ($p<0.05$) (Table 6).

The analysis of the relative expression of ApoB gene was associated with fat traits by the Pearson method. The results showed that relative expression levels of ApoB gene in each tissue were not significantly correlated with fat traits (Table 7).

Table 5 – Correlation analysis of relative expression of FATP1 gene in different tissues and fat traits

Traits		Sebum	Fat	Liver	Muscle
Live weight	R	0.193	-0.604	0.629	0.609
	P	0.756	0.281	0.255	0.276
Sebum thickness	R	-0.329	-0.519	0.95	0.458
	P	0.589	0.370	0.013	0.438
Comb weight	R	0.109	-0.579	0.725	0.628
	P	0.862	0.306	0.166	0.257

a R = correlation coefficient, P = probability.

Table 6 – Relative expression of the ApoB gene in Daweishan Mini chicken

Tissue	Days/d				
	28	49	70	91	112
Sebum	1.000	39.665±19.319	4.221±1.924	38.370±31.222^{a}	8.358±5.658
Fat	1.146±0.436	10.664±6.567	9.723±4.817	6.357±3.223^{a}	1.467±0.593
Liver	633.944±218.334^{A}	5592.086±2265.140	4205.433±1268.103	5230.320±405.672^{bB}	1539.004±337.927^{A}
Muscle	23.005±14.90	115.763±24.661	55.532±20.518	96.525±28.607^{a}	57.019±24.238

^{a,b} Values followed by different lowercase superscripts in the same column are significantly different ($p<0.05$). ^{A,B} Values followed by different uppercase superscripts in the same row are significantly different ($p<0.05$).

Table 7 – Correlation analysis of relative expression of ApoB gene in different tissues and fat traits

Traits		Sebum	Fat	Liver	Muscle
Live weight	R	0.077	-0.276	-0.008	0.130
	P	0.902	0.653	0.990	0.835
Sebum thickness	R	-0.356	-0.371	-0.364	-0.184
	P	0.557	0.539	0.547	0.767
Comb weight	R	0.000	-0.271	-0.051	0.087
	P	0.999	0.660	0.936	0.889

Note: R = correlation coefficient, P = probability.

DISCUSSION

The relationship between ADFP gene expression and fat deposition

The *ADFP* gene was initially detected in fat cells. Brasaemle *et al*. (1997) were the first to determine the mRNA expression of *ADFP* in the lungs, liver, testes, spleen, brain, heart, skeletal muscle, and kidneys of rats. The main cell types in abdominal fat and subcutaneous fat are fat cells,intramuscular fat gradually deposited between muscle bundles as chicken muscle fiber matures (Desruisseaux *et al*., 2007). Therefore, *ADFP* gene in the muscle and adipose tissues is mainly expressed in adipocytes.

The present study revealedsome differences in the *ADFP* gene expression pattern among tissues. *ADFP* gene expression in the abdominal fat and muscle sharply decreased on 91 d, but did notchange on 112 d and 91 d. But the gene expression in the subcutaneous fat was not significantly different between 70 d and 91 d. It appears that *ADFP* gene is regulated by the transcription level, because its the relative expression decreased as adipocytes gradually mature between 70d and 91 d. The maximum relative expression of *ADFP* gene in theliver wasdetected on 70 d. This suggests that liver lipid synthesis was faster when birds were 10 weeks of age, and that the liver may be the main site of triglyceride synthesis in Daweishan Mini chickens.

The relationship between FATP1 gene expression and fat deposition

The fat deposition in different parts of animal body is an important factor affecting the quality of ketone bodies (Hug *et al*., 2004). Fatty acids transmembrane transport is an important prerequisite for fat deposition in poultry, and FATP1 is an important carrier protein involved in fatty acid transmembrane transport (Kubota *et al*., 2002). At present, many experiments have confirmed that FATP1 can stimulate adipocytes to absorb fatty acids (Stahl, 2004; Doege *et al*., 2005; Lobo *et al*., 2007). Studies have shown *FATP1* is highly expressed in skeletal muscle, heart, white adipose tissue (WAT) and brown adipose tissue (BAT) (Schaffer *et al*., 1994; Stahl *et al*., 2002), but has low expression in the brain, kidneys, lungs, liver and keratinocytes (Schmuth *et al*., 2005). The present study showed differences among tissues in *FATP1* gene expression. The expression of this gene in subcutaneous fat did not change on 70 d and 91 d, and sharply declined on 112 d. It appears that *FATP1* gene expression during adipocyte growth (70 d - 91 d) reached the highest level, increasing the rates of fatty acid transport and triglyceride synthesis in the adipose tissue, as well as the amount of fat deposition. However, the relative expression of the *FATP1* gene decreased, as shown by its transcription level during the adipocyte maturation period (on 112 d). The expression of this gene in the abdominal fat and muscle did not change on 49 d and 112 d, indicating that fat deposition was relatively stable at the stage.

Subcutaneous fat thicknessindicates further fat deposition, including in chickens. The relative expression of the *FATP1* gene in the liver was significantly correlated with subcutaneous fat thickness ($p<0.05$). This indicates that *FATP1* gene expression

is closely related to the development of the adipose tissue in Daweishan Mini chickens. Therefore *FATP1* gene developmental expression may reflect fat content changes in the adipose tissue.

Relationship between ApoB gene expression and fat deposition

The protein *ApoB* is not only a key component of VLDL particles and microsomal triglyceride transfer protein (MTP) (Malaguarnera *et al*., 2013), but it isalso the main structural component of triglycerides (TG) secreted by liver (LDL) and present in the intestinal lumen (chylomicron) (Davidson *et al*., 2000; Fisher *et al*., 2002; Hussain *et al*., 2005; Shelness *et al*., 2005). *ApoB* is a glycoprotein that contains 4536 amino acid residues in chickens (Davis *et al*., 2001), and plays an important role in lipid metabolism. *ApoB* gene expression influences energy absorption and reproductive performance, which may directly or indirectly affect fat deposition and growth and development (Sen *et al*., 2006; Zhang *et al*., 2007). The present study showed that the relative expression of the *ApoB* gene was different among tissues and significantly higher in liver than in the other tissues at all stages. Its expression in liver was highest on 49 d, did not change between 70 d and 91 d, and sharply declinedon112 d. This indicates that *ApoB* lipid transport rate in the liver was faster than in the other tissues on 49 d, leading to fat accumulation in these tissues. In addition, maximum fat content affectedfat transport and metabolism on 112 d, reducing fat deposition rate. The pattern of *ApoB*expression in theother three tissues was similar to that in liver, that is, the *ApoB* gene expression in the four tissues as increased and reduced at the same stages. This demonstrates *ApoB* gene expression inthe liver can affect fat deposition in the subcutaneous fat, abdominal fat and muscle.

CONCLUSIONS

This study was the first to demonstratethe expression of the *ADFP, FATP1, APOB*genes in multiple tissues and multiple rearing stages ofDaweishan Mini chickens, as well as the correlation between changes in the expression of these genes and fat traits. Based on the results obtained, we speculate that those three genes have a strong influence on the formation of fat, and may play a role inthe regulation of fat deposition.

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