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Original Article

Study on the Major Genes Related with Fat Deposition in Liver and Abdominal Fat of Different Breeds of Chicken

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ABSTRACT

Fat deposition is higher in fast growing chickens than in slow growing chickens. The liver is the major organ for lipogenesis and fat deposition in chickens, although genetic background, age, and gender also influence fat deposition. In the present study, we aimed to explore the molecular mechanisms underlying fat deposition in liver and abdominal fat. We determined the expression abundances of the key genes regulating fat metabolism in fast-growing (FG) broilers (Cobb) and slow-growing (SG) broilers (HS1) and found that *ACC, FAS, PGC-1α, PPARγ, SREBP-1c* and *PLIN1*genes were expressed in the abdominal fat and liver tissues of FG and SG. ANOVA analysis showed that the breed, age, and tissue factors influenced the expressions of *ACC, FAS, PGC-1α, PPARγ, SREBP-1c*, and *PLIN1* genes in the liver and abdominal fat of FG and SG. Also, the expressions of *PPARγ* and *PLIN1* in the liver of SG were higher than that of FG. The results suggest that the differences in adipocyte development and adipose deposition between breeds are due to genetic factors.

INTRODUCTION

Lipogenesis differs greatly between species. Differences exist at the level of substrate utilization for fatty acid synthesis, tissue site of fatty acid synthesis, and the reducing equivalent generating pathways. Animals usually utilize either glucose or acetate as primary lipogenic substrate (Kuntz *et al.*, 2006; Suagee *et al.*, 2010).

Fatty acid synthesis occurs primarily in either the liver or adipose tissues in most species (Manso Filho *et al.*, 2007). Moreover, distinct adipose tissue depots, such as visceral or subcutaneous, may possess different capacities for lipogenesis. While the liver is the primary site for fatty acid synthesis in humans and avian species, the adipose tissue on the other hand is the primary fatty acid synthesis site for many other animal species (Hirwa *et al.*, 2013; Cui *et al.*, 2018; Mai *et al.*, 2019).

The growth of broiler chicken is accompanied by an increased percentage of body fat with a concomitant increase in the mass of abdominal and visceral fat (Carlborg *et al.*, 2003; Zhao *et al.*, 2011). Fat deposits in chickens contribute significantly to meat quality attributes such as juiciness, flavor, taste and other organoleptic properties. The quantity of fat deposited increases faster and earlier in the fast-growing chickens than in slow-growing chickens (Manso Filho *et al.*, 2007; Claire D'Andre *et al.*, 2013).

Fat deposition is an essential aspect inevitably contributing to chicken breeding because it is directly correlated with the selection for increase body weight in broilers (Zhao *et al.*, 2007b). Fatty acids from poultry are derived from fat breakdown from diets, and fat synthesis in the liver (90~95%) (Jensen-Urstad & Semenkovich, 2012). The

mesenchymal stem cells, derived from the mesoderm, undergo stages of adipogenesis thus, from adipocytes, to preadipocytes and immature adipocytes, and eventually developed into mature adipocytes. In poultry, fat is deposited mainly in the sebum and abdominal fat which then accumulate over time. Animals store the majority of excess dietary energy in the form of lipids. The excess energy of mammals and birds is converted to triglycerides and stored as abdominal fat (Ishimaru *et al.*, 2015). Hence, abdominal fat is formed from the accumulation of metabolic energy. This causes wastage of feed resources following excess fat accumulation in the abdomen. Hence, the current breeder's goals are aimed at controlling excess fat deposition in poultry species (Jiang *et al.*, 2017). Growth rate and body fat percentage are positively correlated, indicating that a significant increase in body weight could unavoidably increase body fat percentage, and correlatively increase abdominal mass and visceral fat (Carlborg *et al.*, 2003; Zhao *et al.*, 2011). Carlborg *et al.*, reported on fat deposition in chickens with fast and slow growth rate characteristics and indicated that fast-growing chicken breeds exhibited high amount of fat deposition as compared to the slow-growing chicken breeds (Carlborg *et al.*, 2003).

Chicken is one of the main animal protein sources which play an important role in the consumption market (Wooming 2015). Therefore, to meet the high demand for chicken products by the increasing human population triggered chicken breeders to focus on improving the quality and quantity of chicken products through set goals and objectives (Rizzi *et al.*, 2009; Imran *et al.*, 2014; Mazzoni *et al.*, 2015), one of which is the regulation of excess fat accumulation. One major problem facing the modern chicken industry is high fat deposition, which negatively affects productivity, acceptability, and health of consumers (Claire D'Andre *et al.*, 2013), this therefore, warrant studies to regulate fat deposition in broiler chicken. Emphasis on regulating fat deposition can significantly contribute to high quality meat. Muscle structure and fat metabolism significantly influence intramuscular fat content and meat quality of animals (Zhao *et al.*, 2007b; Sahraei 2012). Growth is accompanied by an increased protein deposition in broilers with a concomitant increase in muscular weight (Zhao *et al.*, 2011).

Bourneuf et al performed a comparative study on gene expression in fat and lean chicken livers using the microarray analysis method and found that *SREBP-1c* and *ACC* were highly expressed in fat chickens than in lean birds (Bourneuf *et al.*, 2006). Resnyk et al also found that the expressions of *ACC* and *PLIN1* differed significantly in the abdominal fat of chickens with high-growth and low-growth rates chicken breeds (Resnyk *et al.*, 2017). Gene functions were reported to link *ACC* to catalyze the formation of malonyl-CoA, an essential substrate for fatty acid synthesis in lipogenic tissues and serve as a potential inhibitor of fatty acid oxidation (Listed 2009). One of the subunits of Acetyl-CoA carboxylase α (ACCα) was reported to catalyzed biogenesis of long-chain fatty acids and carboxylation of acetyl-CoA to form malonyl-CoA which plays a key role in the regulation of fatty acid metabolism (Tian *et al.*, 2010). *FAS* is a key enzyme which catalyzes the synthesis of fatty acid de novo and has been considered as an anti-obesity target (Liang *et al.*, 2013), whereas, other reports pointed *FAS* to be associated with catalyzing the synthesis of long-chain saturated fatty acid from the substrates of acetyl-CoA (Ac-CoA), malonyl-CoA (Mal-CoA) and NADPH by its seven functional domains arranged in sequence (Schweizer and Hofmann 2004). Furthermore, *PLIN1* was found to influence changes in body weights and fat deposition in animals (Londos *et al.*, 2005; Bickel *et al.*, 2009). The genetic variation of *PLIN1* is associated with carcass traits and adiposity in chickens (Zhou *et al.*, 2014), and exhibited high desire for surfaces of intracellular neutral lipid droplets (Kimmel *et al.*, 2009). *SREBP-1c*, an isoform of *SREBP*, regulates fatty acid synthesis (Horton *et al.*, 1998) and promotes the induction of lipogenic genes to modulate lipid and metabolic derangements (hypertriglyceridemia) (Zhang *et al.*, 2017). *PPAR*γ induce cell growth arrest during differentiation process of fibroblasts to adipocytes (Boitier *et al.*, 2003), and targets genes linked to growth regulatory pathways (Gupta *et al.*, 2001). Inhibiting the expression of *PPAR*γ in *3T3-L1* cells significantly reduced fat synthesis (Ida *et al.*, 2020; Yang *et al.*, 2014). *PGC-1*α is regarded as the potential switch for brown adipocyte differentiation (Pere & Spiegelman 2016), and expressed preferentially in matured brown adipocytes compared to white adipocytes. This shows its involvement in inducing brown fat differentiation (Uldry *et al.*, 2006). In general, fat deposition occurs in subcutaneous tissues, muscles, and the abdomen of chickens (Cui *et al.*, 2018).

Numerous genes involved in the pathway of fatty acid synthesis were significantly expressed in fat birds than in lean ones (Hirwa *et al.*, 2013; Cui *et al.*, 2018; Mai *et al.*, 2019). Genetic mechanisms underlying chicken fat deposition were widely studied for the past decade (Wang *et al.*, 2007). However, past studies have not focused much on the expression of key fat metabolism-related genes in different breeds

of chickens. Therefore, in this study, we determined the molecular mechanisms regulating adiposity in fast-growing (FG) broilers (Cobb) and slow-growing (SG) broilers (HS1) at the individual and cellular levels.

MATERIALS AND METHODS

Animal management and sample collection

This study was approved by the Institutional Animal Care and Use Committee of Sichuan Agricultural University with the permit number DKY-S2018102013. The animals used in this experiment were cared for under the guide-lines stated in the Guide for the Institutional Animal Care and Use Committee of Sichuan Agricultural University of Sichuan province.

In this experiment, two stocks (fast-growing (FG) broilers (Cobb) and slow-growing (SG) broilers (HS1)) with different genetic backgrounds were used. SG were selected from five generations for meat-production at the Poultry Breeding Farm of Sichuan Agricultural University. HS1 originated from the cross between a Hungary Babolna layer and a local breed from Guangdong province in China with a slower growth rate and morphological characteristics such as; black shanks for both sexes, red (male) and yellow (female) plumage, and have strong adaptability and good meat flavor. Cobb broilers were introduced from the Branch Company of Chia Tai Group, Chengdu, China.

A total of 600 quality eggs (300 each for FG and SG) were simultaneously incubated and hatched. Seventy twoSeventy-two chicks of the same average body weights were selected (36 chickens each) and further divided into 3 groups each (with 12 chickens per group). All the chickens were fed in a free-range manner and were fed freely during the feeding process. When the breeding period ends at 28 days of age, all the chickens were transferred to the free-range hen house for continuous feeding until they were 70 days of age. Twelve FG and SG with body weight close to the average body weight were selected for slaughtering at 28, 49 and 70 days of age. Live weight, abdominal fat weight and liver weight of each chicken were weighed and recorded. At the same time, liver and abdominal fat of four chickens at the same site were collected for analysis of gene expression related to fat metabolism during growth of FG and SG. All samples were frozen at -80 °C for quantitative analysis.

Cell Culture and Preadipocytes Differentiation

At 12 days of age, FG abdominal fat was rapidly taken under aseptic conditions as chicken primary preadipocytes were cultured in a total of 25. The abdominal fat tissue was placed in a cell culture dish fitted with PBS, and was rinsed 5 times, to remove visible blood vessels and fascia and then was shred with eye clippers. The shredded tissue was transferred into a 50 mL centrifuge tube and type I collagenase digestion solution was added to digest the tissue at 37°C for 65 min and was shaken thoroughly every 5 min. A complete culture medium was added and then blown with a straw to terminate the digestion process. The supernatant in the discarded tube was added to the erythrocyte lysate, and then blown to resuspend the cells, preceded by incubation at room temperature for 10 min and then centrifuged at 2000 r for 10 min. This was followed by obtaining chicken progenitor preadipocytes by adding an appropriate amount of complete culture medium. The medium was evenly blown and the plates were laid for culturing. The isolated preadipocytes were counted and inoculated into 6-well cell culture plates and T-25 cell culture flasks at a density of 1×10^5 cells/cm^2. Incubation was carried out in a CO_2 incubator at 37°C, 5% and after 24h of incubation, the unattached cells were washed and the culture medium was replaced every 2 days. After attaining 80%-90% confluence of the cell culture, trypsin digester was added for 2 min at 37°C. Thereafter, the complete culture solution was added and blown evenly, and then inoculated in a 1:3 ratio column. After sub-generation, the cells were cultured according to the primary preadipocyte culture protocol, and after the cells attained a confluence again, the secondary sub-generation was conducted.

Determination of fat content by colorimetric method for oil red O extraction

Preadipocytes were inoculated into 6-well plates and the plates were divided into 2 groups: the oleic acid-induced group and the control group, each with 3 wells with a cell density of approximately 1×10^5 cells/cm^2 per well, and was further placed in a CO_2 incubator and changed every 24 h. The cell induction groups were subjected to induction differentiation at 24 h after planking. Thereafter, oleic acid was added and recorded at 0 h, tested at 12 h intervals, and preceded with continuous measurement until 72 h.

CCK-8 cell growth curves

Preadipocytes were inoculated into 96-well plates with a cell density of approximately 1×10^5 cells/cm^2 per well and placed in a CO_2 incubator and the fluid was changed every 24 h. Each plate cell was divided

into 3 subgroups (the oleic acid induced group, the non-oleic acid group, and the cell-free group) with 8 wells taken from each group. The cells of the induction group were induced to differentiate at 24 h after plate laying, and oleic acid addition was recorded at 0 h, and the assay was performed every 12 h for 72 h. This was followed by the addition of 10μL of CCK solution to each well and the 96-well plates were incubated in an incubator for 3 h. thereafter, the absorbance value at 490 nm was determined by an enzyme standardizer. Finally, growth curves were plotted in the horizontal coordinate of time and the OD mean of cells at 490 nm in the vertical coordinate.

RNA Extraction and qRT-PCR

During the periods of end of brood (d 28), market period (d 49) and maximum fat deposition period (d 70), four chickens from each replicate per group were randomly selected, sacrificed, and the liver and abdominal adipose tissues were collected, stored immediately in liquid nitrogen for subsequent RNA extraction with Trizol reagent (TaKaRa) according to the manufacturer's instructions and was further dissolved in DNase/RNase-Free water. The reverse transcription reactions were carried out using the TaKaRa RNA PCR Kit (Takara) and the primer pairs for *ACC*, *FAS*, *PGC-1α*, *PPARγ*, *SREBP-1c,* and *PLINI* mRNA were designed using Premier 5.0 and Oligo 6.0 (Table 1), The kit contains gDNA Eraser with strong DNA decomposition activity, it can remove gDNA and then verified by PCR to ensure removal of gDNA from RNA samples. The 25μL of reaction mixture containing 12.5μL SYBR® Premix Ex Taq II of forward and reverse primer, 1μL, cDNA 2μL, and DNase/RNase-Free water 8.5μL. The extension phase of the PCR consisted of an initial denaturation step at 95 °C for 5 min, followed by 39 cycles at 95 °C for 5s, annealing temperature for 30s, and 72 °C for 10s. The mRNA levels of *ACC*, *FAS*, *PGC-1α*, *PPARγ*, *SREBP-1c,* and *PLIN1* were expressed as concentrations relative to GAPDH mRNA.

Statistical Analysis

The gene expression values were given as the means ± standard error (n = 4 replicates). Statistical comparison was performed by one-way analysis of variance (ANOVA) and multivariate analysis of variance (GLM) using SPSS 20.0 software. In table 3, we adopt the GLM model:

$$Y_{yjk}=\mu + B_i + D_j + T_k + (BD)_{ij} + (BT)_{ik} + (DT)_{jk} + (BDT)_{ijk} + e_{ijk},$$

Where Y_{ijk} = the expression of genes in breed I , day-old j and tissue k, B_i=the effect of breed i (i=1 and 2;SG and FG), D_i=the effect of day-old j (j=1, 2 and 3;28d, 49d and 70d),T_k=the effect of tissue k (k=1 and 2;liver and abdominal fat), $(BD)_{ij}$=the interaction effect of breed i × day-old j, $(BT)_{ik}$=the interaction effect of breed i × tissue k, $(DT)_{jk}$= the interaction effect of day-old j × tissue k,$(BDK)_{ijk}$= the interaction effect of breed i × day-old j × tissue k, e_{ijk}= the random residual effect. When interactions were significant, means were tested by Tukey-Kramer multiple comparisons. *p* values of less than 0.05 (two-tailed) were considered significant.

Table 1 – Sequence of oligonucleotide primers used for real-time quantitative PCR (qRT-PCR).

Gene	Forward primer	Reverse primer	Annealing temperature
GAPDH	AGGACCAGGTTGTCTCCT GT	CCATCAAGTCCACAACACGG	57.0
ACC	CCGAGAACCCAAAACTACCAG	GTCTGAGCCACTATTGACGAC	57.0
FAS	ACGATTACCCGTCTCAATGAAGTT	CCAGGCTCTGTATGCTGTCCAA	54.5
SREBP-1c	TTGGGTCACCGCTTCTTCG	CAGGAGGTGCTCACGGAAGA	58.0
PPARγ	CAGGAAAGACGACAGACAAATCAC	CTGCCTCCACAGAGCGAAAC	51.8
PLIN1	CAAAGCCAAAGGGCAGGAAC	CCTGCAATGCTCTGCCTCTCA	57.0
PGC-1α	CGCAGGTCTTGTTCCCGTTCT	TGTGCTCGGTGTCTACAGTGGC	53.9

RESULTS

Performance and carcass characteristics of FG and SG chicken breeds

The results showed that the SG recorded a slower growth rate as compared to FG. Table 2 showed that the body and liver weights of FG were significantly higher compared to SG at d 28, 49, and 70. The body, liver, and abdominal fat weights of both species increased with increasing age. FG recorded significantly high abdominal fat weights at all-time points (d 28, 49, and 70) as compared to SG, however, there were no significant differences between the two strains. Whereas, the ratio of abdominal fat to body weights showed an opposite trend, recording higher ratios for SG than FG at all-time points, with no significant differences between the two breeds.

Oleic acid induced preadipocytes differentiation and proliferation

In Figure 1, we observed small fat droplets at the middle of the cell at the induction periods of 24 h and

Table 2 – Carcass traits compared between FG and SG chickens.

Breeds	Age (g)	Body weight (g)	Liver weight (g)	Abdominal fat Weight (g)	Abdominal fat Weight/body Weight (%)
FG	28d	828.25±49.94*	29.23±7.42*	5.27±0.99*	0.65±0.10 NS
SG	28d	378.33±11.24	8.60±3.37	4.47±2.88	1.18±0.76
FG	49d	2101.25±60.25*	42.70±2.18*	10.18±2.67 NS	0.48±0.13 NS
SG	49d	983.50±97.63	19.45±1.86	5.73±1.41	0.57±0.10
FG	70d	3687.25±529.77*	61.45±15.86*	22.45±17.26 NS	0.55±0.40 NS
SG	70d	1794.75±73.77	30.53±1.80	14.45±4.51	0.81±0.27

*represent significant differences between the two breeds, NS = No Significant differences.

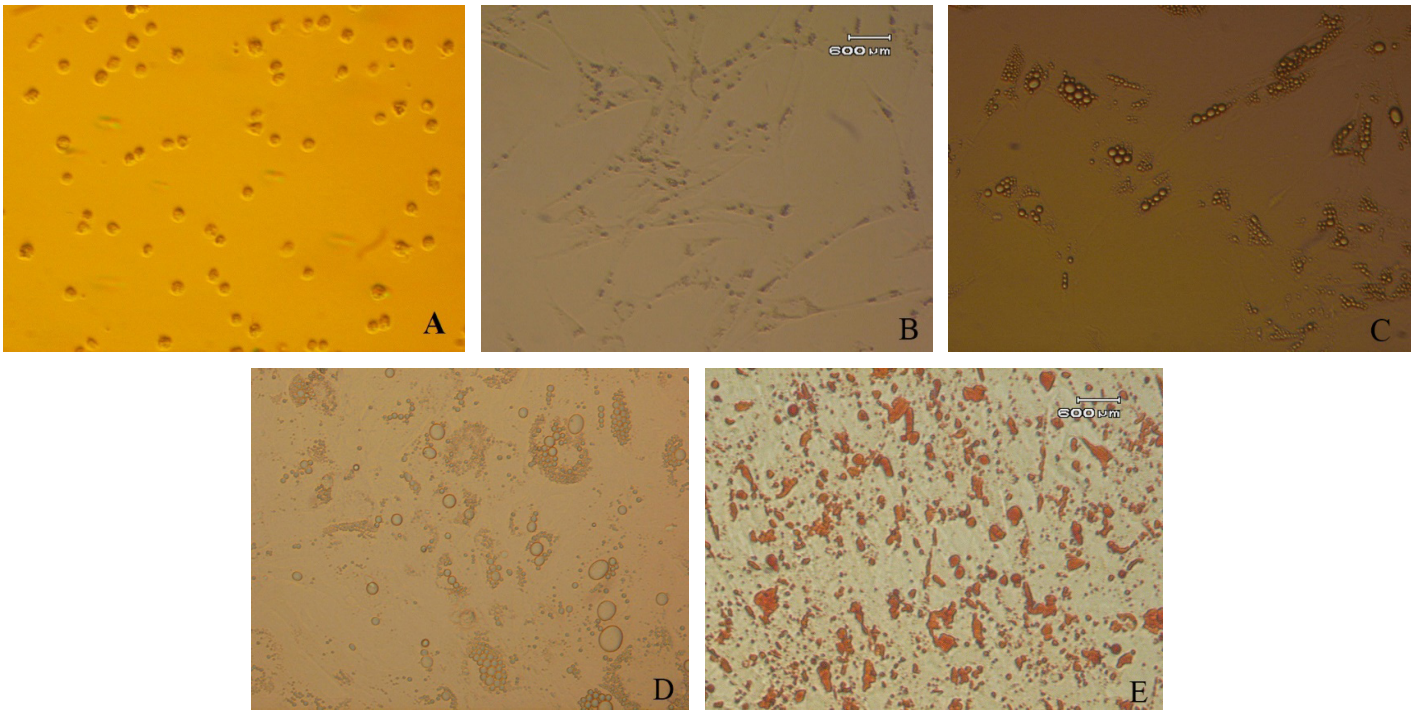

Figure 1 .– Morphological observation of preadipocytes differentiation.

Part A, B, C and D successively exhibited preadipocytes differentiation after they were cultured with oleate for 0, 24, 48, and 72 h. Part E showed lipid droplets in the mature adipocytes after oil red O staining

48 h, while the intracellular portion contained large lipid droplets but at 72 h of induction, grape shaped lipid droplets appeared in the cells and fused into one big fat droplet causing the nucleus to have one sided orientation in the cell.

In this study, qualitative test was performed with CCK method to determined cell growth number (Figure 2A). We observed that at 36 h of induction, the speed of preadipocytes proliferation reached the peak and the total number of cells was observed at 60 h of induction. This observation showed that oleic acid treatment stimulated preadipocytes differentiation and proliferation compared with the control group. Figure 2B showed the qualitative results of oil red O staining

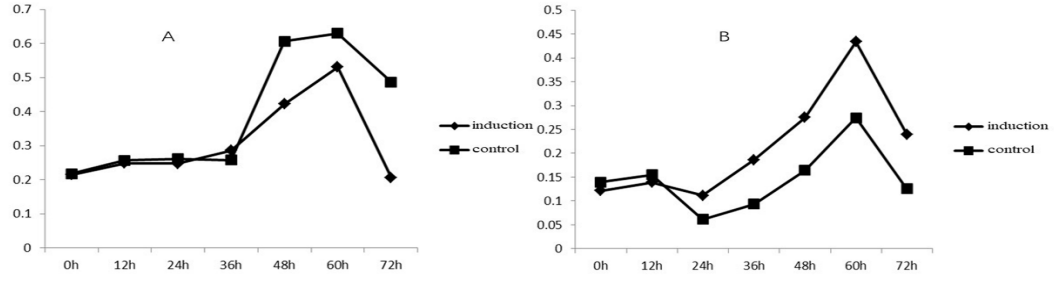

Figure 2 – Induction of preadipocyte to adipocyte by oleate.

Part A showed cell growth curve by CCK-8 test. Part B showed fat content determination by oil red O staining.

determination method. We observed that at 24 h of induction, the intracellular fat content showed a sharp increase and reached its maximum at 60 h of induction, while at 72 h of induction, the intracellular fat content decreases and the fat contents of the induction group still exceeded the control group.

Expression of the six candidate genes in preadipocytes differentiation

The changes in the expression of these major genes (ACC, FAS, PGC-1α, PPARγ, SREBP-1c, and PLIN1) were examined at 24 h, 48 h, and 72 h of induction (Figure 3). During the differentiation process, it was observed that most of the candidate genes initially showed upward expressions and then decreased except PGC-1α and PLIN1 in the control group and FAS in the experimental group. Genes expressed at 24 h of induction showed no significant differences but at 48 h of induction, ACC, PLIN1, and SREBP-1c were significantly higher (p<0.05) in the experimental group except PGC-1α. At 72 hours, except for PGC-1α, the experimental group was significantly higher than the control group, and the induction efficiency was the highest.

Expression of the six candidate genes in FG and SG

Table 3 presented in the results showing the significance of the main effects of breeds, organs, ages, and their interactions. It can be seen from the table that ACC, FAS, PGC-1α and PPARγ did not have

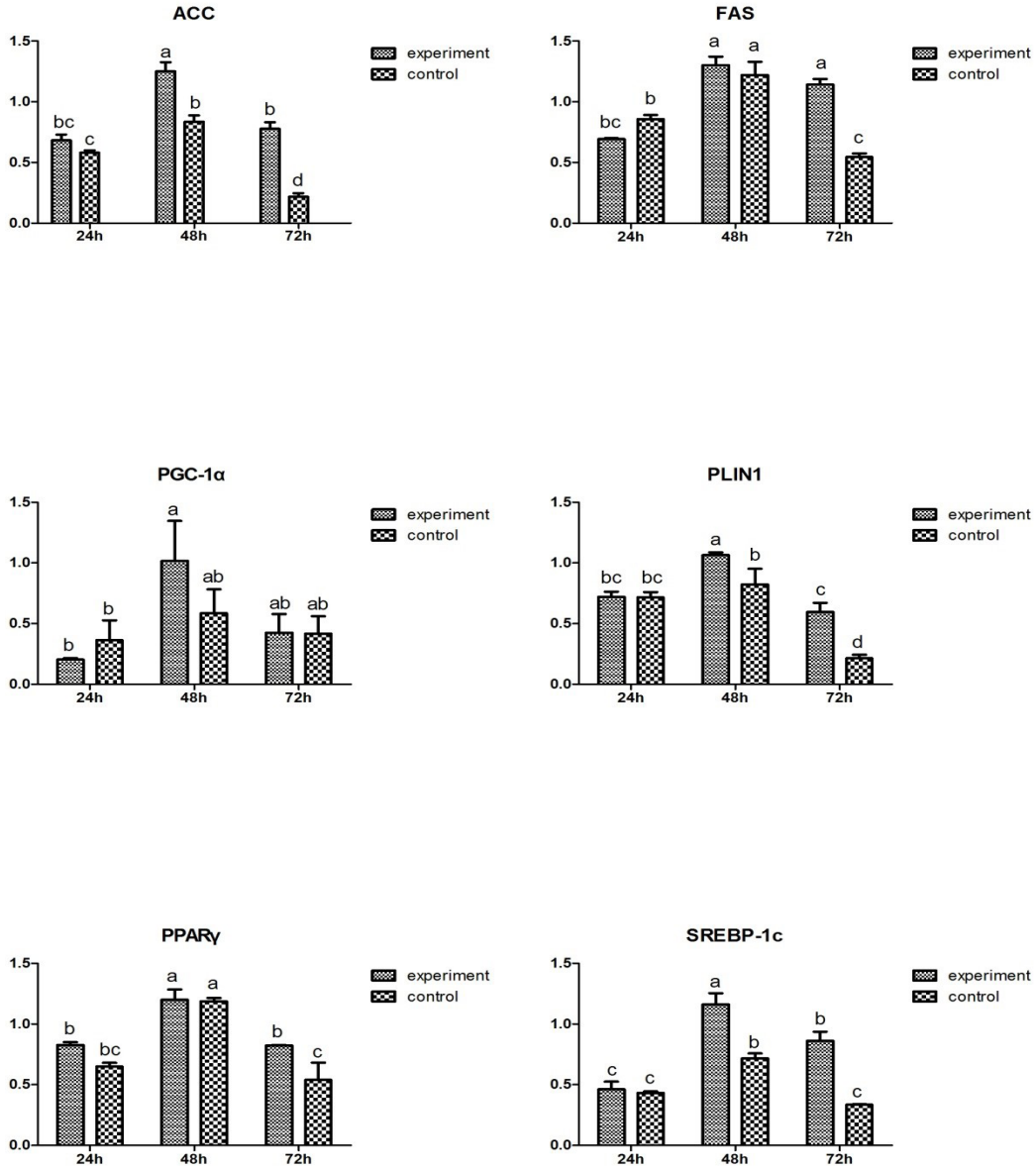

Figure 3 – The expression of ACC, FAS, PGC-1α, PPARγ, SREBP-1c, and PLIN1 during the process of preadipocytes differentiation. In the graph corresponding to each gene, having the same letter indicates that there is no significant difference between them.

Table 3 – Least squares means and SE for traits by age, organ and breed, and breed.

	Genes					
Item	ACC	FAS	PGC-1α	PPARγ	SREBP-1c	PLIN1
Age(d)						
28	1.08±0.17	1.42±0.45	0.44±0.05ab	45.71±5.16	5.30±0.46a	4114.43±637.70a
49	0.63±0.16	1.89±0.41	0.36±0.049b	27.48±4.99	3.54±0.44b	2123.35±599.01b
70	1.26±1.26	1.63±0.44	0.52±0.05a	36.04±5.33	2.94±0.47b	2970.09±618.66ab
p-value	NS	NS	NS	NS	**	*
Organ and Breed1						
FGL	0.63±0.19b	1.43±0.48ab	0.52±0.06b	1.33±5.89c	2.11±0.52b	3.71±683.95b
SGL	0.41±0.19b	0.46±0.46b	0.88±0.05a	1.17±5.62c	1.49±0.50b	3.54±683.95b
FGA	0.95±0.20b	2.40±0.50a	0.16±0.06c	58.59±6.15b	6.01±0.55a	5960.21±714.36a
SGA	2.10±0.20a	2.68±0.54a	0.16±0.06c	95.39±6.15a	6.93±0.55a	7725.39±771.60a
p-value	**	NS	**	**	NS	NS
Breed						
FG	0.78±0.14b	1.89±0.35	0.34±0.04b	28.41±4.26b	3.93±0.38	2825.09±494.49
SG	1.16±0.14a	1.43±0.36	0.53±0.040a	43.58±4.17a	3.96±0.37	3274.50±515.55
p-value	*	NS	**	**	NS	NS

[a–c]Within a source, means with the same superscript are not different.

[1] FGL and SGL represent liver tissues of FG and SG, respectively; FGA and SGA represent abdominal adipose tissues of FG and SG, respectively.

*$p<0.05$; **$p<0.01$.

a significant effect on different days. On d 28, SREBP-1c and PLIN1 were significantly higher than on d 49. Among the effects of these genes on the interaction of organs and breeds, we found that PLIN1 and PPARγ are highly expressed in abdominal adipose tissue, but very low in liver tissue, almost not expressed. FAS, SREBP-1c and PLIN1 have no significant effect on the interaction., InIn abdominal adipose organs, the expression of SG in ACC and PPARγ was significantly higher than that of FG. Among different breeds, the expression levels of ACC, PGC-1α, and PPARγ in SG were significantly higher than those in FG, while FAS, SREBP-1c, and PLIN1 had no difference between the two different breeds.

DISCUSSION

In this study, liver, and abdominal tissues from the two breeds were used. Fat deposition in the liver is in correlation with muscle development in chicken (Cui *et al.*, 2018). Growth in chicken is accompanied by an increased fat and protein deposition resulting in an increased muscular weight (Zhao *et al.*, 2011). The liver is an important organ responsible for the synthesis and metabolism of fatty acids in poultry. The adipose tissue of chicken is limited in synthesizing fatty acids with most de novo fatty acids (70%) originated from the liver (Na *et al.*, 2018). Moreover, the liver is the main site for de novo fatty acid synthesis, in young chicks (Kan *et al.*, 2010; Suagee *et al.*, 2010; Hirwa *et al.*, 2013). Also, excess energy is converted to triglycerides and stored in the abdominal fat (Ishimaru *et al.*, 2015). The strategy of selective fat deposition-related genes in animals is aimed at promoting and enhancing production efficiency and meat quality in chickens (Ricoult & Manning, 2012; Hirwa *et al.*, 2013; Ishimaru *et al.*, 2015). Therefore, there is a close correlation between adipose tissue and liver in terms of triglyceride aggregation which has a positive effect on musculature development (Wang *et al.*, 2010). Numerous efforts including genetic selection, feeding strategies, housing, and environmental strategies were made with the aim of attenuating fat deposition in animals (Dodson *et al.*, 2010).

In this study, the liver weights increased with increasing age between FG and SG groups, but conversely, the ratios of the liver and body weights of FG showed initial decreasing trend but increased upon reaching 7 weeks. This development indicated that in the first 4 to 7 weeks, the liver growth rate was higher than the body weight. In the SG breed, the liver growth rate rises and sharply falls upon reaching 4 weeks of age. This is an indication that the liver growth rate has direct correlation with the muscle or body development, because the liver serves as the site for fat deposition and metabolism in chickens. Moreover, these differences obtained could be linked to the triglyceride contents of the liver. Hepatic triglyceride contents are higher in FG than in SG during embryonic development (Alvarenga *et al.*, 2011). Therefore, this may be related to the higher fat synthesis in the liver of FG than in SG in our study. Also, in our results, the body and liver weights were also correlated with the findings of previous works that reported on the role of fatty

acid-binding protein in the intramuscular trafficking of long-chain fatty acids within intramuscular adipocytes and found that their levels were different among chicken species (Saez *et al.*, 2009; Mai *et al.*, 2019).

Few studies have focused on global gene expression surveys in chickens. Wang *et al.*, provided analysis of chicken adipose tissue gene expression profile (Wang *et al.*, 2007). Other hepatic transcriptional analyses had been reported, using dedicated chicken liver-specific microarray (Cogburn *et al.*, 2004; Xu *et al.*, 2013). Differential gene expressions in the liver and abdominal fat tissues during fat developmental and deposition stages in FG and SG were related to lipid or fat metabolism in our study.

It has been reported that some genes for instance, long chain acyl-CoA thioesterase, fatty-acid elongation enzymes and cytosolic fatty-acid and acyl-CoA-binding proteins plays key roles in lipid metabolism (Ashrafi *et al.*, 2003). Glyco-metabolism such as fat synthesis (*FAS* and *ACC*), fat transduction (*SREBP-1c* and *PPARγ*), fat differentiation (*PGC-1α*) and coated lipid droplets (*PLIN1*) were observed in this study. Genes that expressed significant differences in this study are mostly lipid metabolism-related among which the differences reported between *PPARγ* and *PLIN1* were noticeable in playing vital roles in fat metabolism. *PPARγ* is a prerequisite for adipocyte differentiation (Kajimura *et al.*, 2008). Collin et al reported that FG developed excessive adiposity and high muscle mass after hybrid selection (Collin *et al.*, 2009). This gives an indication that high intramuscular fat guarantees high quality meat in FG breeds compared to SG. Another study suggested that high intramuscular fat guarantees high quality meat in animals (Dodson *et al.*, 2010). The results obtained in this study, suggested that differential expression of the lipid metabolism-related genes might be one of the major factors resulting in the differences of fat deposition and muscle buildup between FG and SG during the developmental stages. Growth rate and fat deposition are directly proportional to muscle development. Zhao et al also explained that growth is accompanied by an increased protein deposition in broilers with a concomitant increase in the muscular weight (Zhao *et al.*, 2011), as observed in this study. We suggested that the expression of genes related to cholesterol biosynthesis, carbohydrate metabolic and fatty acid biosynthesis may have influenced the differences between fat and muscle developments in the two chicken breeds.

Another report indicated that *PPARγ* knockout in mice due to lack of *PPARγ* in the embryonic stem cells led to embryonic death due to non-fat formation (Lefterova *et al.*, 2014), therefore, the differences observed in the expression of *PPARγ* and *PLIN1* genes in this study between the two chicken types, resulted in the differences observed in their fat deposition and muscle buildups. This showed that *PPARγ* and *PLIN1* play key roles in lipid metabolism. This finding is in accordance with other reports that suggest that differences in body weights and fat deposition in animals are influenced by *PLIN1* (Londos *et al.*, 2005; Bickel *et al.*, 2009). Genetic variation of PLIN1 is associated with carcass traits and adiposity in chickens (Zhou *et al.*, 2014), and also exhibits high desire for surfaces of intracellular neutral lipid droplets storage (Kimmel *et al.*, 2009). Several mechanisms modulated by PPARγ or SREBP-1c regulates intracellular non-esterified fatty acids composition, including fatty acid transport, acyl CoA synthetases, fatty acid elongases, desaturases, neutral and polar lipid lipases, and fatty acid oxidation. *SREBP-1c* is responsible for modulating genes related to fatty acid and triglyceride metabolism (Hirwa *et al.*, 2013; Cai *et al.*, 2009). Together, these mechanisms control hepatic lipid composition and thence, affect whole-body lipid composition that significantly contributed to an increase body weight between different chicken breeds (Jump *et al.*, 2005), as reported in this study. The major site of lipogenesis in birds is the liver rather than the adipose tissue (Cai *et al.*, 2009; Hirwa *et al.*, 2013), this is because the liver is the main site for fatty acid biosynthesis. These fatty acids are then transported to the adipose tissue for storage, which resulted in weight gain as reported in this study. These tasks are accomplished through the generation of triglycerides by the liver from fatty acids and L-a-glycerophosphate, packaged into very low-density lipoproteins (VLDL), and then, secreted into the blood. The triglycerides in VLDL are processed by the adipose tissue and finally deposited in the central vacuole of the adipocyte (Jump *et al.*, 2005) which also contributes in muscle buildup.

In this study, we found the expression levels of the candidate genes between the chicken types in the preadipocytes at selected three-time points. The oleate induced preadipocytes differentiation, provides the microenvironment for cell proliferation and differentiation. After the induction periods, intracellular lipid increased greatly, and the expression of genes regulating fat metabolism were accompanied by intracellular fat contents. This showed that an increased intracellular lipids and intramuscular fats promote muscle development and meat quality

comparatively, between the two chicken types. This is in accordance with Zhao et al who reported that muscle structure and metabolism significantly influenced meat quality by increasing intramuscular fat content (Zhao *et al.*, 2007a).

The results obtained after quantitative analysis in this study, showed that individual genes such as *PPARγ* and *PLIN1* have low expressions in the liver compared to the abdominal fat, and certainly showed no significant differences among the two chicken types, but other genes showed trends of high expressions in the abdominal fat except for *PGC-1α*. This result is in line with previous studies which reported that, fatty acid synthesis (*FAS*) occurs during periods of excess energy and concomitantly its gene expression is down-regulated during starvation in the liver, which is the major site for lipogenesis in avian species (Jensen-Urstad & Semenkovich 2012; Hirwa *et al.*, 2013).

We also observed that all the candidate genes studied have no differential expression in the liver and the abdominal fat at all measured time points. This observation is inconsistent with other studies (Bourneuf *et al.*, 2006; Resnyk *et al.*, 2017) that reported differences in some fat metabolic related genes between lean and fat animals and/or correlated to adipose tissue weight and could play important roles in the regulation of adiposity in chickens. This observation could be due to specie differences in this study and the previous studies. The above results further confirmed that the *ACC*, *FAS*, *PGC-1α*, *PPARγ*, *SREBP-1c,* and *PLIN1* genes are related to fat deposition in broiler chickens.

CONCLUSION

In conclusion, the results of this study showed that the expressions of *ACC*, *FAS*, *PGC-1α*, *PPARγ*, *SREBP-1c,* and *PLIN1* genes were significantly affected by species, age, and tissue. Therefore, differential gene expressions in FG and SG show differences in fat developmental stages which is supported by lipid-related genes identified and characterized in these two types of chickens. The findings in this study also indicated that the variations in the expression of *ACC*, *FAS*, *PGC-1α*, *PPARγ*, *SREBP-1c,* and *PLIN1* genes were significantly associated with fat deposition and muscle buildup, meanwhile, the expression between *PPARγ* and *PLIN1* gives a clear difference between FG and SG in fat metabolism. Further studies on proteomics are required to explore the protein expressions of these genes.

CONFLICTS OF INTEREST

The authors declare no conflict of interest.

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