CORRELATIVE ANALYSIS OF LIPID CONTENTS AND RELATIVE GENE EXPRESSION BETWEEN CHICKEN BREEDS

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The muscle composition of fatty acid has appeared as economically important traits due to their profound effect in meat quality. It has been shown that breed and age are most important factors effect on the muscle fatty acid composition in chickens. However, the molecular mechanism of fatty acid composition between the local Chinese chicken and commercial broiler which showed different growing rate is still unclear. To address the molecular mechanism underlying of muscle fatty acid composition, we comparatively analyzed the fatty acid (FA) compositions in breast and thigh muscle with different ages by using an Agilent 6890 Series EGC system gas chromatograph equipped with a HP Innowax (30 m × 0.32 mm inside diameter) cross-linked polyethylene glycol column and FID detector (Agilent Technologies, USA) in Chinese native Wuding chickens and fast-growing Cobb broiler. Furthermore, quantitative reverse transcription PCR (Polymerase Chain Reaction) (RT-qPCR) was performed to investigate expression pattern of related candidate genes of fatty acid composition including D9D (Delta-9-Desaturase), FADS-2 (Fatty Acid Desaturase-2), FASN (Fatty Acid Synthetase), FATP-1 (fatty acid transport protein-1) and LPL (lipoprotein lipase) in relative breast and thigh muscles. The results showed that Wuding chickens showed both higher levels of SFA and USFA in two type muscles and two time points due to higher muscle lipid contents compare with Cobb broiler. Thus, Wuding chickens had approximately twice to three folds amount of MUFA (monounsaturated fatty acid) and PUFA (polyunsaturated fatty acid) and association with higher abundance expression of FADS-2, D9D and FATP-1 mRNA in two type of muscles and at two time points compare to broilers. In contrast, broiler showed low amount of MUFA and PUFA and association with higher abundance expression LPL mRNA in two type muscles and at two time points. Similar with reports in fish, rat and human, the FADS-2 and D9D gene code protein might played important role in operation as Δ6 or Δ9 desaturases to synthesis USFAs, including Oleic acid (C18:1), Linoleic acid (LA, C18:2n-6), a-Linolenic acid (ALA, C18:3 n-6) and Docosahexaenoic acid (C22:6n-3, DHA) in chicken muscles. Breed, age and muscle type had significantly influence on the muscle fatty acids composition and expression profiling of related lipid metabolism or fatty acid synthase gene. It is obviously that FADS-2, D9D, FATP-1, FASN and LPL genes play important role in regulating muscle fatty acid synthase and influence in meat quality in chicken.

Keywords: Chicken breeds, age, fatty acid contents, gene expression.

INTRODUCTION

Undesirable healthy effects of saturated and trans fatty acids (FAs) have gained attention of consumers regarding the quantity and types of dietary fat in the muscle (Dhaka et al., 2011). However, polyunsaturated FA (PUFA) and monounsaturated FA (MUFA) are recognized as acknowledged as favorable for human health (Burlingame et al., 2009). The enhanced ratio of MUFA to saturated FA (SFA) and the melting point are influenced from FAs composition of meat that increase fat softness, thus boost the palatability. Moreover, increasing MUFA content improves the quality and nutritional value of animal products (Brooks et al., 2011; Gamarra et al., 2018). Lipid metabolism or biosynthesis mainly occur in the liver of chickens in which synthesis of lipids take place in adipose tissues. Lipids, after synthesis are transported via blood circulation in the form of lipoproteins to specific tissues, where lipoprotein lipase (LPL) hydrolyzes the lipoproteins and fatty acids are released for immediate utilize deposition (Wang et al., 2017). There are different factors that affected on fatty acid composition and fat percentage in particularly including nutritional, genetics and age factors that contribute most difference in composition of fatty acids (Scollan et al., 2006). The different gene expression or enzyme activity that involved in fatty acid synthesis cause the fatty acid composition differences between breeds (De Smet et al., 2004; Scollan et al., 2006). Age is most important factor that
affecting fatty acid composition among the non-nutritional factors. The content of muscle fat and subcutaneous tissue enhance when age progressing however, the proportion of PUFA vs SFA declines (Warren et al. 2008). This is the reason that often consumers have interpretation that older animal meat tend to have more fat and unfit for human health and consumption (Kelava Ugarković et al., 2013).

Numerous findings reported that fast-growing chickens due to muscular-skeletal problems and low motor activity are not habitual to extensive rearing conditions (Dal Bosco et al., 2012). LPL causes the hydrolysis of plasma lipoproteins, which is a rate-limiting step in the lipid transportation into peripheral tissues (D’Andre et al., 2013). Fatty acid synthase (FASN) is an important multifunctional enzyme contributing in the fatty acid synthesis. Some findings revealed the positive correlation between FASN mRNA expression level and body fat content in animals like pigs and rats (Laliotis et al., 2010). However the molecular mechanism of expression pattern of candidate genes in breast and thigh muscles including D9D, FADS-2, FASN, FATP-1, LPL and FA contents comparison between local Chinese Yunnan chicken breed Wuding and Cobb broiler is poorly understood. In current study, the molecular mechanism and characterization of mRNA expression profiles in the breast and thigh muscles of 12 and 16-weeks-old Wuding and Cobb broiler chickens were performed by the genetic selection of five genes that could influence on breast and thigh muscles quality with increase of age.

MATERIALS AND METHODS

Animals and diets: In present study, Wuding (local native breed of Yunnan province of P. R. China) and the Cobb broiler chickens were procured from Yunnan Agricultural University chicken farm and Kunming Zhengda Group (Kunming, Yunnan, P. R. China). A total of 100 chickens, 50 from each breed were kept under standard conditions on a starter diet as period I (20.6% CP & 12.8 MJ/kg ME) to 30 d and after 30 days a regular diet as Period II (18.4% CP & 12.5 MJ/kg ME) was fed till the end of trial as shown in the Table 1. Diet contents were consistent with the formulation to meet NRC 1994 (NRC, 1994) and Chinese Chicken Feeding Standard and (2004) recommendations. The same light regime and temperature were provided in all the experimental period and one-day-old chickens were reared in floor pens in the environmental control shed. For the first two days, the brooding temperature was maintain at 35°C and then lowered gradually to 22°C (45% relative humidity) until the end of experiment.

Slaughter procedure and sample collection: All chickens used in this work were permitted from Animal Care Committee, Yunnan Agricultural University (Yunnan, China). Twenty five chickens from each breed of similar body weight were chosen and slaughtered according to National Experimental Animal Slaughter Standard of China by cervical dislocation at 12 and 16 weeks age. Thigh and breast muscle samples were excised and preserved in cryopreservation tubes in liquid nitrogen after that to storage at -80°C until further use. Moreover, the meat samples from the breast and thigh muscles were taken, freeze-dried and used to determine the fatty acid contents.

Table 1. Composition of the diet for the Period I and Period II (g/kg, air dry) used in the experiment

<table>
<thead>
<tr>
<th>Dietary Component</th>
<th>Period I</th>
<th>Period II</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maize</td>
<td>545.0</td>
<td>580.0</td>
</tr>
<tr>
<td>Soy protein</td>
<td>190.0</td>
<td>167.0</td>
</tr>
<tr>
<td>Toasted soybean</td>
<td>140.0</td>
<td>80.0</td>
</tr>
<tr>
<td>Fish meal</td>
<td>35.0</td>
<td>20.0</td>
</tr>
<tr>
<td>Wheat bran</td>
<td>30.0</td>
<td>100.0</td>
</tr>
<tr>
<td>Soya oil</td>
<td>25.0</td>
<td>18.0</td>
</tr>
<tr>
<td>CaHPO₄·2H₂O</td>
<td>12.4</td>
<td>12.4</td>
</tr>
<tr>
<td>Stone meal</td>
<td>11.7</td>
<td>11.5</td>
</tr>
<tr>
<td>Lysine</td>
<td>2.2</td>
<td>2.2</td>
</tr>
<tr>
<td>Methionine</td>
<td>1.6</td>
<td>1.6</td>
</tr>
<tr>
<td>Salt</td>
<td>2.1</td>
<td>2.3</td>
</tr>
<tr>
<td>Minerals and vitamins mix²</td>
<td>5.0</td>
<td>5.0</td>
</tr>
<tr>
<td>Metabolism Energy (MJ/Kg)</td>
<td>12.8</td>
<td>12.6</td>
</tr>
<tr>
<td>Crude protein (CP)</td>
<td>205.5</td>
<td>183.5</td>
</tr>
<tr>
<td>Crude fat</td>
<td>54.1</td>
<td>56.5</td>
</tr>
<tr>
<td>Calcium</td>
<td>10.5</td>
<td>9.8</td>
</tr>
<tr>
<td>Available phosphorus</td>
<td>6.8</td>
<td>6.5</td>
</tr>
<tr>
<td>Lysine</td>
<td>13.5</td>
<td>12.1</td>
</tr>
<tr>
<td>Methionine + Cysteine</td>
<td>8.8</td>
<td>7.8</td>
</tr>
</tbody>
</table>

1: Period I is age 1-30 days; Period II is older than 30 days of age; 2: Supplied per kilogram of diet: antioxidant, 100 mg; biotin, 0.3 mg; vitamin A, 12,000 IU; vitamin D₃, 3000 IU; vitamin E, 18.75 mg; vitamin K₃, 2.65 mg; vitamin C, 12.6 mg; cyanocobalamin, 0.025 mg; folic acid, 2.2 mg; niacin, 35 mg; pyridoxine, 6 mg; riboflavin, 9 mg; thiamine, 3.0 mg; choline chloride, 600 mg; Co, 0.3 mg; Cu, 12 mg; Fe, 50 mg; I, 1 mg; Mn, 125 mg; Mo, 0.5 mg; Se, 200 µg; Zn, 60 mg.

Fatty acids contents: Extraction of FAs was done from the breast and thigh muscles and methylated according to the mentioned as (Moran et al., 2018), except that n-hexane replaced benzene for extraction. The FAs analysis was performed on an Agilent 6890 Series EGC system gas chromatograph equipped with a HP Innowax (30 m × 0.32 mm inside diameter) cross-linked polyethylene glycol column and FID detector (Agilent Technologies, USA). The samples were performed under the following operating conditions: injector and detector temperature 250°C, programmed column temperature changed from the beginning 220°C, nitrogen gas flow 5 ml/min, hydrogen 40 ml/min, air 450 ml/min. Identification of FAs were done by comparison of their retention times with that of the internal standard (C17:0, Fluka 51633). The concentration of FAs are expressed as
percentage of the sum of total identified peaks measured in every sample.

**RNA extraction, cDNA and RT-qPCR:** Using the Basic Local Alignment Search Tool (BLAST) from the National Center for Biotechnology Information (http://www.ncbi.nlm.nih.gov/BLAST) specific primers were established. Primers of genes including D9D, FADS2, FASN, FATP1 and LPL were synthesized commercially (Shanghai Shenggong Biotechnology Company P. R. China). Tissues were homogenized. RNA extraction and RT-qPCR as previously described were performed (Dou et al., 2017; Jia et al., 2018; Talpur et al., 2018). β-actin was used as a reference gene. The sequence of the primer, products length, accession numbers and relevant annealing temperatures are shown in Table 2.

**Table 2. Primer sequence for the target genes and their annealing temperatures.**

<table>
<thead>
<tr>
<th>Genes</th>
<th>Sequence of Primers (5'-&gt;3')</th>
<th>Annealing Temperature (°C)</th>
<th>Product length (bp)</th>
</tr>
</thead>
<tbody>
<tr>
<td>β-actin</td>
<td>F: TGGACTCTCTAACACAAAGGG</td>
<td>59.7</td>
<td>258</td>
</tr>
<tr>
<td>D9D</td>
<td>F: TCATCAGTCCGCTTCTTG</td>
<td>59.7</td>
<td>193</td>
</tr>
<tr>
<td>FADS-2</td>
<td>F: TGGAGACAGACAGACAGACA</td>
<td>59.7</td>
<td>202</td>
</tr>
<tr>
<td>FASN</td>
<td>F: TGGAGTCATGTTGCAAGCC</td>
<td>58.2</td>
<td>213</td>
</tr>
<tr>
<td>FATP-1</td>
<td>F: TCACCCACAACTCAGATCCC</td>
<td>58.2</td>
<td>183</td>
</tr>
<tr>
<td>LPL</td>
<td>F: TGCTGGGAAAAGAGTGTGTCG</td>
<td>60.0</td>
<td>229</td>
</tr>
</tbody>
</table>

**Statistical analysis:** The least square means and standard error of the means (SEM) were applied on all tables. The gene mRNA abundance in tissues among chickens at the same age and among different ages within a breed/hybrid were determined by using SPSS 22.0 (IBM Corp, Armonk, NY), t-test and data were normalized in Excel. Statistical significance of difference at an age labeled by * for P < 0.05, and ** for P < 0.01 while within a breed with different ages is labeled by lower-case letters for P < 0.05 and capital letters for P < 0.01.

**RESULTS AND DISCUSSION**

**Fatty acids contents in breast and thigh muscles:** Poultry meat production is playing a crucial role in the food economy and supplying the protein source for human consumption that is based on rearing high-producing commercial strains of broiler, under environmentally controlled conditions that results in prompt growth rate, high dressing percentage and heavy breast and thigh muscles (Zdunczyk & Jankowski, 2013). Due to poultry meat consumption and requirements, dual purpose chicken production is of marginal significance and obtaining meat from layer chickens is no alternatives to intensive broiler production, but allocate an opportunity to make full use of the conserved breeds in organic or backyard farms (Padhi, 2016).

The basic energy source and indispensable components for metabolism and cellular regulation in animals are fatty acids that play critical roles in meat nutrition, sensory, tenderness and taste flavor (Warren et al., 2008). Several findings indicated that genetic factor play an important role muscle fatty acid composition in pigs (Muñoz et al., 2013; Lee et al., 2016; Zhang et al., 2016; Ballester et al., 2017), in beef cattle (Zhang et al., 2015), in lamb (Rovadoscki et al., 2018) and in chickens (Franco et al., 2012; Jin et al., 2018). In agreement with these reports, the present study showed that dual purpose production medium-growing Wuding chicken and fast-growing the Cobb broiler breed at same environment have different biological mechanisms activities and fatty acid profiles both at Weeks 12 and 16. Compare with Cobb broiler, Wudging chickens showed both higher levels of SFA and USFA in two type muscles and at two time points due to higher muscle lipid content as well as high intramuscular fat (IMF) (Li, 2018). Thus, Wudging chicken had approximately twice to three folds amount of MUFA and PUFA both two type muscle and two time points (Table 3 & 4). Meat containing high intramuscular fat (IMF) is contemplated to possess better taste, conferring juiciness to the meat. Fatty acid (FA) composition of IMF affects the meat nutritional and sensory quality parameters (Puig-Oliveras et al., 2016). This is a reason why local slow-growing native breed showed better taste than fast-growing broilers (Li, 2018). Due to high lipid content in muscles, Wudging chicken showed high individual fatty acids levels in SFAs, MUFAs and PUFAs as well. Especially for MUFAs and PUFAs, Wudging showed higher C16:1(n-7); C17:1(n-7), C18:1(n-9); C18:2(n-6); C18:3(n-6); C20:2(n-6); C20:4(n-6) ; C20:5(n-3) and C22:6(n-3) (P<0.05 or P<0.01) both in two type muscles and two time points (Table 3 & 4). The differences in the genetic background of these breeds determine the IMF and its FA composition, affecting meat quality (Puig-Oliveras et al., 2016). PUFAs contain more than two double bonds and are a critical nutrients group that regulates brain development and cognition as well as diseases including cancer, cardiovascular disease and diabetes (Park et al., 2015). Twenty-carbon PUFAs are precursors of eicosanoids that modulate immune and inflammatory responses through pro and anti-inflammatory activities. Moreover, docosahexaenoic acid (DHA, 22:6N-3) is a precursor of anti-inflammatory docosanoids (Cardoso et al., 2016; Puig-Oliveras et al., 2016). It is obvious that Wuding chicken showed high meat quality association with high muscle IMF, MUFAs and PUFAs compare to broiler. It has been reported that genotypes selected on the basis of precocity and ability to reach marketing live weight at an early age, are not appropriate with longer rearing periods. Sirri et al., (2010) described the lipid composition in three different strains of

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chickens (slow growing Brown Classic Lohman strain, medium-growing NN strain and the fast growing Cobb 700 strain the fast growing Cobb 700 strain), were reared on organic conditions, exhibited an enhanced lipid contents of the fast-growing strain (Sirri et al., 2010).

### Table 3. Determination of fatty acids in breast muscles of different chicken breeds (Freeze-dried samples, unit: mg/g).

<table>
<thead>
<tr>
<th>Fatty acids</th>
<th>Wuding chicken 12 Week</th>
<th>Wuding chicken 16 Week</th>
<th>Broiler chicken 12 Week</th>
<th>Broiler chicken 16 Week</th>
</tr>
</thead>
<tbody>
<tr>
<td>C12 : 0</td>
<td>0.07 ± 0.002</td>
<td>0.01 ± 0.001</td>
<td>0.01 ± 0.001</td>
<td>0.00 ± 0.001</td>
</tr>
<tr>
<td>C14 : 0</td>
<td>0.13 ± 0.003</td>
<td>0.13 ± 0.002</td>
<td>0.13 ± 0.002</td>
<td>0.12 ± 0.002</td>
</tr>
<tr>
<td>C16 : 0</td>
<td>0.08 ± 0.016</td>
<td>0.08 ± 0.016</td>
<td>0.08 ± 0.016</td>
<td>0.08 ± 0.016</td>
</tr>
<tr>
<td>C18 : 0</td>
<td>0.14 ± 0.013</td>
<td>0.14 ± 0.013</td>
<td>0.14 ± 0.013</td>
<td>0.14 ± 0.013</td>
</tr>
</tbody>
</table>

**Note:** The comparison of fatty acid contents in breast muscles between the different ages of the different chickens. Statistical significance of difference at an age labeled by * for P < 0.05, and ** for P < 0.01 while within a breed with different ages is labeled by lower-case letters for P < 0.05 and capital letters for P < 0.01.

### Table 4. Determination of fatty acids in thigh muscles of different chicken breeds (Freeze-dried samples, unit: mg/g).

<table>
<thead>
<tr>
<th>Fatty acids</th>
<th>Wuding chicken 12 Week</th>
<th>Wuding chicken 16 Week</th>
<th>Broiler chicken 12 Week</th>
<th>Broiler chicken 16 Week</th>
</tr>
</thead>
<tbody>
<tr>
<td>C12 : 0</td>
<td>0.028 ± 0.002</td>
<td>0.017 ± 0.003</td>
<td>0.050 ± 0.008</td>
<td>0.020 ± 0.01</td>
</tr>
<tr>
<td>C14 : 0</td>
<td>0.872 ± 0.065</td>
<td>0.135 ± 0.013</td>
<td>1.405 ± 0.013</td>
<td>1.040 ± 0.013</td>
</tr>
</tbody>
</table>

**Note:** The comparison of fatty acid contents in thigh muscles between the different ages of the different chickens. Statistical significance of difference at an age labeled by * for P < 0.05, and ** for P < 0.01 while within a breed with different ages is labeled by lower-case letters for P < 0.05 and capital letters for P < 0.01.

Significant differences of fatty acid composition between breast and thigh muscles were observed in both breeds. There are significantly higher amounts of SFA and USFA in thigh muscles than that of in breast muscle due to higher muscle lipid content as well as high intramuscular fat in thigh muscles. Similarly, it has been shown there are increased trend for individual fatty acid composition varies with breed and age in thigh muscles compare to breast muscle both in two breeds. Our results agree with previous reports that there are significant differences for fatty acid compositions between the breast and thigh muscles (Puchala et al., 2015; Jin et al., 2018). Furthermore, age or growing period also play important role in regulating muscle fatty acid composition. Broiler showed an increasing trend of fatty acids deposition increasing with age in two type muscles. In agreement with reports that chickens had high accumulation of fat in muscle increasing with age or high feed intake (Dal Bosco et al., 2012). In contrast, Wuding chickens showed a decreasing trend of fatty acids deposition increasing with age in two type muscle. It might be explained that Wuding chickens deposited more fat in subcutaneous or belly result in decreased fat or fatty acids deposition in muscle increasing with age (Li, 2018). Similarly, it has been showed there are increased trend for individual fatty acid compositions for broiler increasing with age in two type muscle while the opposite results were observed in Wuding chickens (Table 3, 4 & 5).
Chicken breeds & analysis of lipid contents

Table 5. Determination of fatty acids contents in breast and thigh muscles of different chicken breeds (freeze-dried samples, unit: %).

<table>
<thead>
<tr>
<th>Fatty acids</th>
<th>12 Week</th>
<th>16 Week</th>
<th>12 Week</th>
<th>16 Week</th>
<th>12 Week</th>
<th>16 Week</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Wuding chicken</td>
<td>Broiler chicken</td>
<td>Wuding chicken</td>
<td>Broiler chicken</td>
<td>Wuding chicken</td>
<td>Broiler chicken</td>
</tr>
<tr>
<td>SFA/TUFA</td>
<td>31.48</td>
<td>35.97</td>
<td>37.25</td>
<td>27.47</td>
<td>37.48</td>
<td>36.79</td>
</tr>
<tr>
<td>MUFA/TUFA</td>
<td>36.80</td>
<td>25.27</td>
<td>32.02</td>
<td>36.99</td>
<td>30.67</td>
<td>27.25</td>
</tr>
<tr>
<td>PUFA/TUFA</td>
<td>31.72</td>
<td>38.76</td>
<td>30.73</td>
<td>35.55</td>
<td>31.84</td>
<td>35.97</td>
</tr>
<tr>
<td>USFA/TUFA</td>
<td>68.52</td>
<td>64.03</td>
<td>62.75</td>
<td>72.53</td>
<td>62.51</td>
<td>63.21</td>
</tr>
<tr>
<td>EFA/TUFA</td>
<td>30.29</td>
<td>36.47</td>
<td>28.75</td>
<td>31.89</td>
<td>30.27</td>
<td>34.29</td>
</tr>
</tbody>
</table>

6: n-3 PUFA ratio, which was slightly lower that young broiler chickens (Žlender et al., 2000) that was lower twice in native Spanish hen breeds muscle (Franco et al., 2012) and identical as to slow and medium growing chicken varieties (Dal Bosco et al., 2012). Castellini et al., (2002) reported that there have a concurrent increase of PUFA a and SFA in muscle of organically reared broiler chickens at age 81 d that also verify our study (Castellini et al., 2002). According to our study, thigh muscle of broiler has more PUFA (35.97%) at age of 12 and 16 weeks that has close similarity in results of (Sohaib et al., 2017) that susceptibility to oxidative degradation, leading to lower consumer acceptability for broiler meat products while in contrast in native chicken breed the Wuding, PUFA of thigh and breast muscles decreases with increase of age.

Lipid regulating genes in breast and thigh tissues

FADS-2 (Fatty Acid Desaturase-2) and mRNA gene expression: By catalyzing fatty acyl desaturases (FADS), originating of a double bond at a specific position of acyl chain and have been nominated according to as ∆6, ∆5, ∆4 and ∆8 desaturases (Meesapyodsuk & Qiu, 2012). The biosynthesis of biologically active 22:5n-6 and 22:6n-3 PUFAs in all organisms were considered to occur via ∆4 desaturation (Park et al., 2015). Docosahexaenoic acid (DHA) is a limiting highly unsaturated fatty acid (HUFA) and in neural tissues is a ∆4-desaturated C22 fatty acid. The biosynthesis of Δ4-desaturated docosanoids fatty acids 22:6n-3 and 22:5n-6 are imagined proceeding via a circuitous biochemical pathway requiring repeated use of a FADS-2 protein to perform Δ6 desaturation on C24 fatty acids in the endoplasmic reticulum followed by 1 round of β-oxidation in the peroxisomes. In the present study, Wuding chickens showed significantly higher expression of FADS-2 mRNA in two type muscles than that of Cobb broiler chickens at 12 weeks (P < 0.05) and 16 weeks (P < 0.01) (Fig. 1 & 2) and association with higher amount of 22:6n-3 (Docosahexaenoic acid, DHA) in two type muscles and two time points (Table 3 & 4).
In agreement with report in human that the FADS-2 gene product catalyzes Δ4 desaturation to yield n-3 docosahexaenoic acid and n-6 docosapentaenoic acid in human cells (Park et al., 2015). In agreement with reports in rabbit fish (Siganus canaliculatus) (Li et al., 2010), teleost fish (Solea senegalensis) (Morais et al., 2012; Oboh et al., 2017), pike silverside (Chiromostoma estor) (Fonseca-Madrigal et al., 2014) and striped snakehead (Channa striata) (Kuah et al., 2015), human cells (Park et al., 2015) and human plasmas (Tabassum et al., 2018), the FADS-2 gene code protein might play important role in operation as Δ6 or Δ9 desaturases to synthesis PUFAs including alpha-linolenic acid (C18:3 n-3), linoleic acid (C18:2 n-6), docosahexaenoic acid (C22:6n-3), dihomo-gamma-linolenic acid (C20:3 n-6) in chicken muscles.

**D9D (Delta-9-Desaturase) and mRNA gene expression:** In the Wuding chickens at 12 and 16 weeks (Fig. 3 & 4), D9D mRNA expression in breast and thigh muscles showed higher expression than that of in broiler as agreement with these reports (Miya et al., 2003; Berg et al., 2007) that delta-9-desaturase or stearoyl-CoA-desaturase (SCD) is an enzyme that regulates the biosynthesis of MUFAs, PUFAs, phospholipids, triacylglycerol, wax and choleseryl which is submerged in the endoplasmic reticulum membrane, initiate a cis-double bond exclusively at the delta 9 position of acyl-CoA. D9D transforms stearic acid to oleic acid, which is essential for cell membranes synthesis. Indeed, in present study, Wuding chickens showed higher amounts of MUFAs and PUFAs in muscles as compared to broiler. Similarly, Wuding chickens showed higher amounts of Oleic acid (C18:1), linoleic acid (LA, C18:2n-6) and a-Linolenic acid (ALA, C18:3 n-6) in two type muscles association high abundance expression of D9D gene mRNA in relative muscles.

**FASN (Fatty Acid Synthetase) and mRNA gene expression:** Fatty acid synthase (FASN) is the key enzymes essential for the anabolic conversion of dietary carbohydrates to fatty acids and a multifunctional homodimeric protein. The FASN is the fundamental enzyme in de-novo lipogenesis, catalysing the conversion of malonyl-CoA into palmitate which causing the excess energy intake metabolic consequences and fat mass increased. The FASN synthesizes long-chain fatty acids from three substrates: acetyl-CoA as a primer, malonyl-CoA as a 2 carbon donor, and NADPH for reduction. The FASN plays crucial role in the development of obesity and body weight regulation (Kovacs et al., 2004). As shown in (Fig. 5 & 6), no significant different expression of FASN mRNA were measured between two breeds at week 12 in two type muscles (P>0.05). Broiler showed significant difference in (P < 0.05) expression of FASN mRNA in breast muscle while the contrary result was measured in thigh muscle at 16 weeks showed significantly higher (P<0.01) as compared to Wuding chickens. For Wuding chickens, a local fat chicken breed with high abundance FASN mRNA in thigh muscle at 16 Weeks in agreement with these reports that FASN gene expression high in human adipose tissues contributes to the obesity development (Berndt et al., 2007). The gene expression of FASN is significantly higher in obese as compared to lean individuals, supporting the results that adipocyte size is correlated with FASN levels and expression (Shimokawa et al., 2002; Blüher et al., 2004). In contrast to these report in Diraison et al. (2002) found that FASN mRNA expression was lowered in the subcutaneous adipose tissue of obese vs lean individuals (Diraison et al., 2002). This result supported our measurement for Broiler that showed higher abundance FASN mRNA in breast muscle at 16 weeks. Differences in
species, tissue, nutritional status, metabolic parameters might explain these divergent findings.

Similarly, this difference may be due to energy consumption and fat storage during the development would be the difference between two breeds at same age. Recent findings reported profound weight loss and food intake reduced due to inhibition of FASN in rodents, suggesting that FASN may be involved in obesity through energy homeostasis and regulation of feeding behaviour (Berndt et al., 2007). Cui et al., observed the developmental expression pattern of FASN in three fat related organs including liver, thigh and breast of a slow-growing (Beijing-you) and fast-growing (Arbor Acres). They found that the expression of FASN gene mRNA in the liver were significantly higher than those in breast and thigh muscles and no significant difference for expression of FASN mRNA were measured from 21d to 90 d in both breast and thigh muscles in Beijing-You and Arbor Acre chickens (Cui et al., 2012). These results revealed that there is positive correlation between FASN gene expression and fat synthesis in the liver or adipose tissue. The physiological function of FASN in skeletal muscle whether revolving synthesis of fat is still unclear.

**FATP-1 and mRNA gene expression:** FATP-1 belongs to the fatty acid transport protein (FATP) family; the first of this protein family to be identified and best characterized in adipocytes (Pohl et al., 2004). It has been described before that insulin enhances FATP-1 translocation from an intracellular compartment to the plasma membrane and concomitantly increases Esterified long-chain fatty acids (LCFA) uptake (Stahl et al., 2002). The FATP-1 involved in the transmembrane transport of FAs and metabolism that effects on body fat contents (Binnert et al., 2000). In present study, Wuding chickens showed significant difference in expression (P < 0.05) of FATP-1 mRNA for breast muscle at 12 week and thigh muscle at 16 week compared to broiler (Fig. 7 & 8). These observations were consistency with skeletal muscle FA compositions in present study and in previous study for body fat composition (Li, 2018). Because FATP-1 can indirectly effect on lipid profile and deposition by regulating FA uptake and metabolism in the skeletal muscles, the determination of expression may be important for genetic improvement of meat traits (Pohl et al., 2004).
Consistently, gain-of-function studies have shown that FATP-1 increases FA import into adipocytes (Choi et al., 2011). Moreover, decreasing FAs uptake in skeletal muscle and adipose tissue with reduced mass and plasma FA level elevates in case of loss of FATP-1 (Wu et al., 2006). Similar with reports in human, mice or pigs, FATP-1 played an important role in influence lipid profile and FA deposition in chickens.

**LPL (lipoprotein lipase) and mRNA gene expression:** The LPL enzyme is responsible for catalyzing the triglycerides, caused by FAs and glycerol circulation synthesized by adipocyte level (Hossner, 2005). The adipocyte cells are initiated to form when they received lipid accumulation signal (Mead et al., 2002; Hossner, 2005). In contrast to other genes, LPL gene mRNA expression in the broiler was significantly higher (P < 0.01) in breast muscle at 12 and 16 weeks of age compare to the Wuding chickens (Fig. 9 & 10). These observations were consistency with skeletal muscle FAs compositions in present study and in previous study for body fat composition. Thus, compare to local Chinese chickens breed, broiler showed low amount of skeletal muscle FAs compositions, less fat tissue and more lean tissue in carcass (Li, 2018). Lipoprotein lipase (LPL) is mainly expressed in adipocytes and skeletal muscle tissues, and is a crucial enzyme in lipid metabolism (Merkel et al., 2002). There was different expression pattern of LPL gene mRNA between Guangxi hybrid chickens (Sanhuang) and broiler chickens that showed significant different carcass traits (Huang et al., 2016).

Within breed, expression of LPL gene mRNA increasing with age in two type muscles in both breeds (Fig. 9 & 10). Similarly, expression of LPL gene mRNA significantly increased from day 7 to 120, and expression peaked on day 120 both in Guangxi hybrid chickens (Sanhuang) and broiler chickens (Huang et al., 2016). Furthermore, expression of LPL gene mRNA significantly increased with age or growing period in pigs and association with carcass fat tissue deposition (Mooradian & Albert, 1999; Shan et al., 2010).
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and at two time points. Similar with reports in fish, rat and human, the FADS-2 and D9D gene code protein might played important role in operation as Δ6 or Δ9 desaturases to synthesis USFAs, including Linoleic acid (LA, C18:1), Oleic acid (C18:1), α-Linolenic acid (ALA, C18:3 n-6) and Docosahexaenoic acid (C22:6n-3, DHA) in chicken muscles. Breed, age and muscle type had significantly influence on the muscle fatty acid composition and expression profiling of related lipid metabolism or fatty acid synthase gene. It is obviously that FADS-2, D9D, FATP-1, FASN and LPL genes play important role regulating muscle fatty acid synthesis and influence in meat quality in chicken.

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