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Gene expression and enzyme activity of lipoprotein lipase correlate with intramuscular fat content in Guangxi san-huang and Arbor Acres chickens

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ABSTRACT. Lipoprotein lipase (LPL) is a key enzyme in lipid metabolism. This study investigated *LPL* gene expression, LPL enzyme activity, and the correlation of each with intramuscular fat (IMF) in Chinese Guangxi san-huang (GXSH) and Arbor Acres (AA) chickens. The results showed that age and breed had significant effects on *LPL* expression and enzyme activity. Correlation analyses showed significant positive correlations between *LPL* expression levels and IMF contents in the breast and thigh tissues of both GXSH ($r = 0.712$, $P = 0.001$; $r = 0.792$, $P < 0.001$, respectively) and AA ($r = 0.644$, $P < 0.001$; $r = 0.545$, $P < 0.001$, respectively) chickens. The results also indicated a significant positive correlation between LPL enzyme activity and IMF contents in the breast and thigh tissues of both GXSH ($r = 0.615$, $P = 0.001$; $r = 0.685$, $P < 0.001$, respectively) and AA ($r = 0.600$, $P = 0.001$; $r = 0.528$, $P = 0.003$, respectively) chickens. The results indicated that the *LPL* gene was significantly correlated with IMF in these two breeds. The results presented here could contribute to knowledge of

LPL mRNA developmental expression patterns and enzyme activity, and it could facilitate further research on the molecular mechanisms underlying IMF deposition in chickens.

Key words: Chicken; Lipoprotein lipase gene; Meat quality; Intramuscular fat

INTRODUCTION

Intramuscular fat (IMF) is an important evaluation index of meat quality, which affects pork color, flavor, tenderness, and water-holding capacity (Chambaz et al., 2003; Gao and Zhao, 2009). A certain amount of IMF can provide the favorable meat taste sought by consumers. Therefore, exploration of the molecular mechanisms underlying IMF deposition plays an important role in meat regulation.

Some studies have examined the molecular mechanisms associated with excellent meat quality in mammals such as Laiwu (Hu et al., 2008) and Jinhua (Guo et al., 2011), which are local Chinese breeds. Reports showed that IMF deposition was associated with functional genes such as *H-FABP* (Nechtelberger et al., 2001), *Sirt1* (Moynihan and Imai, 2006), etc. Until now, compared to monogastric mammals, little was known about the molecular mechanisms associated with excellent meat quality in the Guangxi san-huang chicken (GXSH chicken). This breed is known for its good meat quality, which is indicated by its tenderness and juiciness.

Lipoprotein lipase (LPL) is mainly expressed in adipose and skeletal muscle tissues (Merkel et al., 2002), and it is a key enzyme in lipid metabolism (Maingrette and Renier, 2003). Some studies have examined the relationship between IMF contents and *LPL* mRNA expression levels in the mammals such as pigs (Shan et al., 2006). Furthermore, previous studies also suggested that LPL in abdominal adipose tissue plays a crucial role in fat accumulation in broiler chickens (Sato et al., 1999). Using high-resolution melting curves, Zhang et al. (2015) showed that *ADSL* (adenylosuccinate lyase) and *LPL* gene mutations were correlated with meat quality differences in GXSH chickens and ISA B-line layers. However, knowledge of the developmental expression patterns of *LPL* mRNA, LPL enzyme activity, and the relationship of each with IMF contents in GXSH chicken is limited.

In the present study, the developmental expression patterns of *LPL* mRNA, LPL enzyme activity, and the correlation of each with IMF was detected using two fat-related tissues (breast and thigh) from a slow-growing Guangxi local breed (GXSH) and a fast-growing commercial broiler line (Arbor Acres, AA). Determination of the expression patterns of LPL mRNA and LPL enzyme activity would provide a basis for further studies of lipid metabolism, especially the molecular mechanisms associated with IMF deposition in chickens.

MATERIAL AND METHODS

Animals and sample collection

Two hundred 1-day-old GXSH chickens and 200 1-day-old AA chickens were used as experimental animals. The animals were maintained at the Base For Teaching and Practice in the College of Animal Science and Technology at Guangxi University. The experiments were conducted in parallel using the same diets and management conditions. The ingredient

and chemical compositions of the diets during the starter phase (1-20 days) was as follows: CP (crude protein) ≥ 20.0%, CF (crude fiber) ≤ 8.0%, ASH (Ash) ≤ 10.0%, lys (lysine) ≥ 0.9%, Ca (calcium) 0.8-1.3%, P (phosphorus) ≥ 0.60%, and NaCl (sodium chloride) ≥ 0.3-0.6%. The diets during the grower phase (after 20 days) were as follows: CP ≥ 19.0%, CF ≤ 5.0%, ASH ≤ 9.0%, EE (ether extract) ≥ 3.0%, effective lys ≥ 1.0%, Ca 0.7-1.3%, and P ≥ 0.50%. Water was provided *ad libitum* during the experiment. All experimental procedures were performed under the Guidelines of Animal Experimentation outlined by the Committee of Experimental Animal Care, Guangxi University, China.

At each sampling time (GXSH: days 7, 28, 56, 70, 90, and 120; AA: days 7, 14, 21, 28, 35, 42, and 56), three female birds and three male birds from each breed with similar weights were killed via stunning and exsanguination 12 h after the last meal. Breast and thigh samples were obtained postmortem, and were packed into vials. The samples were then frozen in liquid nitrogen and stored at -80°C until further processing.

Measurement of lipid content

Visible fat and fascia were removed from each tissue sample, and the samples were then cut into pieces and labeled as powder. The samples were dried during two 10-12-h stages (65ºC followed by 105°C), and were then cooled in a desiccator for at least 30 min. The IMF contents in breast and thigh samples were measured using the Soxhlet method. The results are reported as percentages based on the dry tissue weight (AOAC, 1990).

LPL enzyme activity

Muscle samples (500 mg) were homogenized in a buffer (10.01 mM Tris-HCl, 0.01 mM sucrose, 0.0001 mM EDTA-Na_2, 0.8% NaCl, 1000 mL formulated solution, pH adjusted to 7.4). Solubles were recovered after centrifugation at 2500 rpm for 10 min at 4ºC. The supernatant was removed using a sterile pipette tip, and it was then placed in another sterile centrifuge tube prior to storage at -80ºC. LPL activity of the tissue samples was quickly measured using an LPL Activity Assay Kit (Bioengineering Institute of Nanjing Jiancheng Company, Nanjing, China), according to the manufacturer protocol.

Total RNA extraction and reverse transcription

Total RNA was extracted from the breast and thigh tissues using Trizol Reagent (Invitrogen Life Technologies, Carlsbad, CA, USA) according to the manufacturer protocol. The purity and concentration of the total RNA was determined using a spectrophotometer at 260 and 280 nm. Transcription was performed with 1.0 μg total RNA by using a PrimeScript™ RT reagent Kit with gDNA Eraser (Perfect Real-Time) (Takara Biotechnology Co. Ltd., Dalian, China).

Real-time PCR

The chicken LPL and GAPDH (glyceraldehyde-3-phosphate dehydrogenase) primers used for the real-time PCR (RT-PCR) were as follows: LPL sense 5'-AGGAGAAGAGGCAG CAATA-3' and antisense 5'-AGCCAGCAGCAGATAAG-3'; GAPDH sense 5'-CTTTCCGTG TGCCAACCCC-3' and antisense 5'-CAGCAGCAGCCTTCACTACC-3'. The specificity of

the primers was detected using melting curve analyses, and the amplification efficiency of the genes was detected using a standard curve. The following formula was used for amplification efficiency:

$$\text{Efficiency} = 10^{-1/\text{slop}} - 1 \qquad \text{(Equation 1)}$$

cDNA was amplified using Takara SYBR Premix Ex Taq under the following conditions: 95ºC for 5 min; 40 cycles for 10 s at 95ºC; and 30 s at 58ºC. A melting curve analysis was conducted to verify the specificity of the reactions after amplification. All results were normalized to GAPDH levels, and relative quantification was calculated using the $2^{-\Delta\Delta Ct}$ method. All samples were run in triplicate, and the average values were calculated.

Data analysis

Data are reported as means ± standard error of the mean (SE). Significant differences between groups in all of the experiments were determined using ANOVA and Duncan-type multiple comparisons, and the SPSS 18.0 biostatistic software was used to perform these analyses. A P value less than 0.05 was considered statistically significant.

RESULTS

Expression of *LPL* mRNA in two chicken breeds

The results of the melting curve analyses indicated that the specificity of the two RT-PCR primers was good, and non-specific amplification was not detected. Further analysis of the standard curve showed that the amplification efficiencies of GAPDH and LPL were 99.3 and 95.4%, respectively (between 90 and 110%). Therefore, the results showed that the primers were in accordance with the requirements of RT-PCR amplification, so they were sufficient for quantitative tests of gene expression. We detected *LPL* expression in the breast and thigh tissues of GXSH and AA chickens at different developmental stages using RT-PCR with specific primer sets (Figure 1). The results of RT-PCR analyses showed that *LPL* mRNA expression levels in GXSH breast and thigh tissues increased with age (Figure 1). In the breast and thigh tissues of GXSH chickens, *LPL* mRNA expression level improved from day 7 to 120, and expression peaked on day 120 (Figure 1A and B). However, the results of RT-PCR analyses showed that *LPL* mRNA abundance in the breast tissues of AA chickens trended upwards during days 7 to 28 (Figure 1C). Expression peaked at 28 days, and then gradually decreased from days 28 to 56 (Figure 1C). *LPL* gene expression in AA thigh tissues increased from days 7 to 28 (Figure 1D). It then gradually decreased from days 28 to 42, and levels increased from days 42 to 56 (Figure 1D). Furthermore, the results showed that sex (female and male) had no significant effect on *LPL* mRNA expression levels for GXSH and AA chickens (Figure 1).

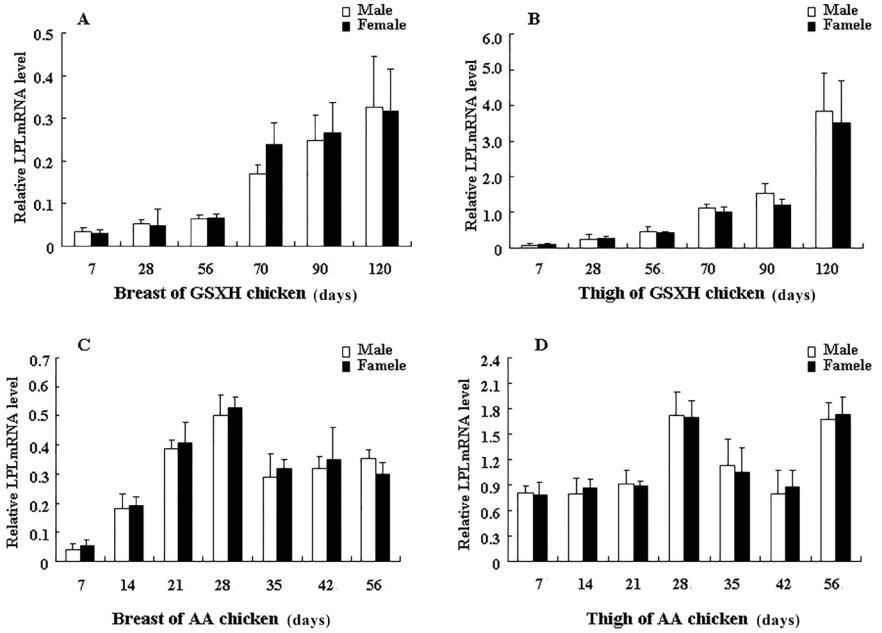

Figure 1. *LPL* gene expression levels based on RT-PCR analyses of different tissues on different days (i.e., ages) for GXSH and AA chickens. **A.** and **B.** show the *LPL* expression levels of GXSH chickens in different tissues (breast and thigh, respectively). **C.** and **D.** show the *LPL* expression levels of AA chickens in different tissues (breast and thigh, respectively).

Comparisons of the two breeds at the same developmental stages indicated that *LPL* expression levels in the breast tissues of GXSH chickens on days 28 and 58 were significantly lower than those of AA chickens ($P < 0.01$ for both; Figure 2), and there was no significant difference ($P > 0.05$) on day 7. Moreover, *LPL* expression levels in the thigh tissues of GXSH chickens on days 7, 28, and 58 were significantly lower than those of AA chickens ($P < 0.01$; Figure 2). However, *LPL* mRNA expression levels in the thigh tissues of GXSH chickens were higher than those of AA chickens at marketing ages (GXSH chicken: day 120; AA chicken: day 56) (Figure 2).

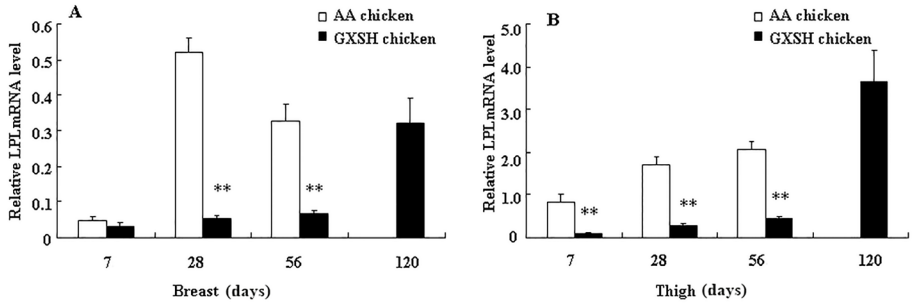

Figure 2. A. LPL gene expression levels in GXSH and AA chickens based on RT-PCR analyses of breast tissues during the same developmental stages. **B.** LPL gene expression levels in GXSH and AA chickens based on RT-PCR analyses of thigh tissues during the same developmental stages. **$P < 0.01$.

LPL enzyme activity in two chicken breeds

We also analyzed the LPL enzyme activity at different developmental stages in the two chicken breeds. The result showed that LPL enzyme activity in the breast and thigh tissues of the two breeds increased with age (Figure 3). LPL enzyme activity in the thigh tissues of these two breeds was significantly higher than that observed in the breast tissues. Moreover, the results showed that sex (female and male) had no significant effect on LPL enzyme activity for GXSH and AA chickens.

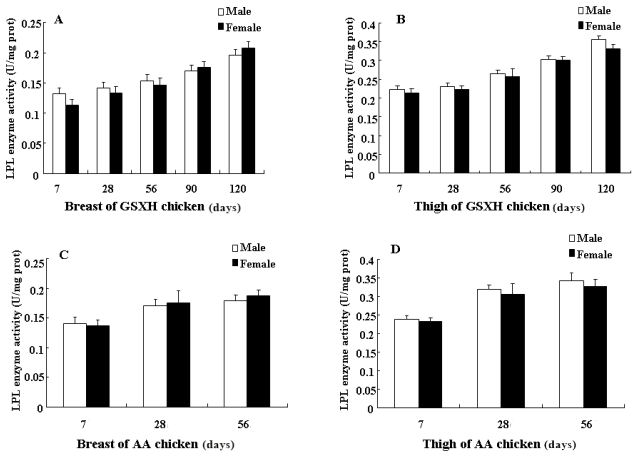

Figure 3. LPL enzyme activity in different tissues of GXSH chickens and AA chickens at different ages. **A.** and **B.** show LPL enzyme activity of GXSH chickens in different tissues (breast and thigh, respectively). **C.** and **D.** show LPL enzyme activity of AA chicken in different tissues (breast and thigh, respectively).

When LPL enzyme activity was compared between the two breeds, we found that the activity in breast and thigh tissues of GXSH chickens on days 28 and 56 was significantly lower than that observed in AA chickens ($P < 0.01$ for both measurements) (Figure 4). However, LPL enzyme activity in the breast or thigh tissues of GXSH chickens was higher than that of AA chickens at the marketing age (Figure 4).

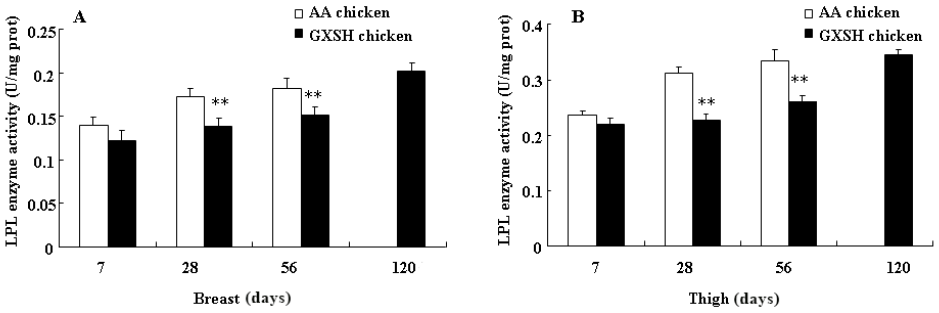

Figure 4. A. LPL enzyme activity in the breast tissues of GXSH and AA chickens during the same developmental stages. **B.** LPL enzyme activity in the thigh tissues of GXSH and AA chickens during the same developmental stages. **$P < 0.01$.

IMF content of different tissues in two chicken breeds

The IMF contents in the breast and thigh tissues of GXSH and AA chickens were measured at different developmental stages (Tables 1 and 2). The results showed that the IMF contents of different tissues increased with age in the two chicken breeds. Furthermore, the IMF content was significantly higher in the thigh tissues than in the breast tissues of both breeds, and this was observed at all developmental stages (Tables 1 and 2).

Table 1. IMF contents of different tissues at different developmental stages in GXSH chickens.

Age (days)	Breast of GXSH chickens (%)			Thigh of GXSH chickens (%)		
	Male	Female	Average	Male	Female	Average
7	1.94 ± 0.03	1.71 ± 0.02	1.81 ± 0.03^{d}	8.55 ± 0.14	7.92 ± 0.15	8.23 ± 0.13^{e}
28	1.99 ± 0.02	2.29 ± 0.03	2.13 ± 0.04^{d}	8.74 ± 0.13	10.05 ± 0.13	9.39 ± 0.18^{d}
56	2.37 ± 0.05	2.51 ± 0.05	2.44 ± 0.04^{cd}	9.67 ± 0.14	9.45 ± 0.11	9.56 ± 0.09^{d}
70	2.64 ± 0.06	2.78 ± 0.10	2.71 ± 0.06^{bc}	11.03 ± 0.18	10.86 ± 0.14	10.94 ± 0.11^{c}
90	2.91 ± 0.20	3.06 ± 0.19	3.00 ± 0.11^{b}	12.14 ± 0.39	12.61 ± 0.32	12.37 ± 0.25^{b}
120	3.52 ± 0.11	3.67 ± 0.47	3.59 ± 0.07^{a}	13.15 ± 0.24	13.88 ± 0.36	13.51 ± 0.23^{a}
		Breast		Thigh		
P value	Age	<0.001		<0.001		
	Sex	NS		NS		
	Age x Sex	NS		NS		

Data are reported as means ± SE. Different lowercase letters indicate significant differences ($P < 0.05$) in each vertical list. NS indicates no significant differences.

Table 2. IMF contents of different tissues at different developmental stages in AA chickens.

Age (days)	Breast of AA chicken (%)			Thigh of AA chicken (%)		
	Male	Female	Average	Male	Female	Average
7	2.12 ± 0.08	1.87 ± 0.07	2.00 ± 0.06^{d}	10.77 ± 0.11	9.64 ± 0.09	10.20 ± 0.15^{c}
14	2.06 ± 0.09	2.13 ± 0.08	2.10 ± 0.06^{cd}	8.06 ± 0.17	8.54 ± 0.10	8.29 ± 0.11^{d}
21	2.44 ± 0.08	2.71 ± 0.08	2.58 ± 0.06^{bc}	10.38 ± 0.15	10.76 ± 0.10	10.57 ± 0.10^{c}
28	3.18 ± 0.12	2.87 ± 0.08	3.02 ± 0.08^{ab}	10.96 ± 0.09	11.86 ± 0.09	11.41 ± 0.12^{b}
35	2.74 ± 0.08	2.82 ± 0.08	2.78 ± 0.06^{ab}	11.07 ± 0.14	11.72 ± 0.09	11.40 ± 0.11^{b}
42	3.05 ± 0.22	3.12 ± 0.31	3.08 ± 0.04^{ab}	11.96 ± 0.20	12.37 ± 0.15	12.16 ± 0.13^{ab}
56	3.23 ± 0.36	3.31 ± 0.26	3.27 ± 0.07^{a}	12.57 ± 0.30	12.93 ± 0.35	12.75 ± 0.15^{a}
			Breast	Thigh		
P value		Age	<0.001	<0.001		
		Sex	NS	NS		
		Age x Sex	NS	NS		

Data are reported as means ± SE. Different lowercase letters indicate significant differences ($P < 0.05$) in each vertical list. NS indicates no significant differences.

The IMF contents in the both tissue types were measured during the same developmental stages in both chicken breeds (Table 3). The results indicated that IMF contents in both tissues on days 28 and 56 were significantly higher ($P < 0.01$) in AA than in GXSH chickens (Table 3). However, at the marketing age, GXSH chickens (day 120) exhibited higher IMF contents than the AA chickens (day 56) (Table 3). Furthermore, the results showed that the fat contents in the AA chicken tissues were significantly higher than observed in the tissues of GXSH chickens during the early development stage. Therefore, the time required for fat deposition in GXSH chickens was longer than that of AA chickens.

Table 3. IMF contents of breast and thigh tissues at the same developmental stages in two chicken breeds.

Age (days)	Breast (%)		Thigh (%)	
	AA chicken	GXSH chicken	AA chicken	GXSH chicken
7	2.00 ± 0.06	1.81 ± 0.03	10.20 ± 0.15^{A}	8.23 ± 0.13^{B}
28	3.02 ± 0.08^{A}	2.13 ± 0.04^{B}	11.41 ± 0.12^{A}	9.40 ± 0.18^{B}
56	3.27 ± 0.07^{A}	2.44 ± 0.04^{B}	12.75 ± 0.15^{A}	9.56 ± 0.09^{B}
120		3.59 ± 0.07		13.51 ± 0.23

Data are reported as means ± SE. Different lowercase letters indicate significant differences ($P < 0.01$) in each lateral list.

Relationship between LPL mRNA expression levels and IMF content

Correlation analyses showed significant positive correlations (Table 4) between *LPL* expression levels and IMF contents in the breast and thigh tissues of both GXSH ($r = 0.712$, $P = 0.001$; $r = 0.792$, $P < 0.001$, respectively) and AA ($r = 0.644$, $P < 0.001$; $r = 0.545$, $P < 0.001$, respectively) chickens. The results further indicated that LPL played a key role in fat synthesis in breast and thigh tissues. These results might provide a theoretical basis for further studies of molecular mechanisms that underlie IMF deposition in chickens.

Table 4. Correlation coefficient between the LPL mRNA expression levels and IMF contents in breast and thigh tissues.

	Correlation coefficients between *LPL* mRNA and IMF content			
	Breast of GXSH chicken	Thigh of GXSH chicken	Breast of AA chicken	Thigh of AA chicken
R value	0.712	0.792	0.644	0.545
P value	0.001	<0.001	<0.001	<0.001

Relationship between LPL enzyme activity and fat contents

We also found a significant positive correlation between LPL enzyme activity and fat contents in the breast and thigh tissues of both GXSH and AA chickens (Table 5). Furthermore, the results indicated a significant positive correlation between LPL enzyme activity and IMF contents in the breast and thigh tissues of both GXSH ($r = 0.615$, $P = 0.001$; $r = 0.685$, $P < 0.001$, respectively) and AA ($r = 0.600$, $P = 0.001$; $r = 0.528$, $P = 0.003$, respectively) chickens.

Table 5. Correlation coefficient between LPL enzyme activity and IMF contents in breast and thigh tissues.

	Correlation coefficient between LPL enzyme activity and IMF content			
	Breast of GXSH chicken	Thigh of GXSH chicken	Breast of AA chicken	Thigh of AA chicken
R value	0.615	0.685	0.600	0.528
P value	0.001	<0.001	0.001	0.003

DISCUSSION

Different livestock have different chemical properties and meat qualities. The GXSH chicken, a type of chicken with "yellow feathers, yellow claws, yellow beak" from the Guangxi Province of China, is especially noted for high-quality meat. On the contrary, the AA chicken is a meat-producing breed noted for increased growth rate, feed efficiency, and low-quality meat. However, the molecular mechanisms that regulate meat quality remain unknown.

Differences in meat quality traits are closely related to gene expression. At present,

researchers recognize that IMF deposition is one of the key factors influencing meat quality. Therefore, researchers succeeded in identifying functional genes associated with IMF deposition in pigs (Shan et al., 2006) and chickens (Sato et al., 1999). For GXSH chickens, little is known about the molecular mechanisms of IMF that are associated with superior quality. LPL is a rate-limiting enzyme that hydrolyzes triglyceride (TG)-rich lipoprotein (van der Vusse et al., 1992). In research on the expression of LPL in muscle tissues, knowledge of the relationship between *LPL* mRNA expression levels and fat contents is expected to improve meat quality, regulate animal body weight, etc. In the present study, we differentially compared the mRNA abundance of *LPL* and LPL enzyme activity as well as the IMF contents in the breast and thigh tissues of Chinese GXSH chickens and the popular western fast-growing meat breed AA chickens. Both RT-PCR and enzyme detection methods were used to determine enzyme activity.

Expression patterns of *LPL* mRNA in two chicken breeds

Since the *LPL* of humans and other animal species were cloned (Wion et al., 1987; Harbitz et al., 1992; Broad et al., 1995), the gene has been a hot topic. Most reports mainly focused on the effects of dietary fats (Takahashi and Ide, 1999), fasting (Spurlock et al., 2001), and refeeding (Bonnet et al., 2000) on *LPL* mRNA expression levels. However, we could not find information regarding *LPL* mRNA expression levels in GXSH chickens.

To understand the differences between *LPL* mRNA expression levels in the two chicken breeds, the *LPL* expression levels were analyzed in the breast and thigh tissues of GXSH and AA chickens at different developmental stages using RT-PCR. As the results indicated, *LPL* mRNA expression levels in the breast and thigh tissues of GXSH chickens increased with age, peaking when the chickens reached marketing age. This result suggested that the change in *LPL* expression at an early developmental stage was consistent with other livestock such as pigs (Duroc x Landrace x Yorkshire) (Shan et al., 2006). Aging is known to have an effect on the expression of some genes expressed in adipose tissue (Mooradian and Albert, 1999). Furthermore, we found that AA chicken breast tissue exhibited an *LPL* mRNA abundance upward trend between 7 and 28 days. Expression then peaked at 28 days, and then gradually decreased from days 28 to 56. *LPL* gene expression in AA chicken thigh tissues increased from days 7 to 28, and then gradually decreased from days 28 to 42, followed by an increase from days 42 to 56. Therefore, the effect of aging on the *LPL* mRNA level seems to be dependent on the animal species and tissues.

When *LPL* mRNA expression patterns were compared between the two breeds, we found that expression levels in the breast and thigh tissues of GXSH chickens on days 7, 28, and 56 were significantly lower than those of AA chickens. However, *LPL* mRNA expression levels in the breast or thigh tissues of GXSH chickens were higher than those of AA chickens at marketing age. This likely resulted because the growth rate of AA chickens is higher than GXSH chickens, so energy consumption and fat deposition during development would differ between the two breeds at the same developmental age.

LPL enzyme activity in two chicken breeds

Previous studies suggested that LPL activity seemed to be a more important factor in the control of fatty acid uptake and intramuscular fattening than LPL expression or LPL protein contents (André et al., 2007). We detected LPL enzyme activity at different developmental

stages in the two chicken breeds using LPL detection methods. The results showed that LPL enzyme activity in the muscle tissues of GXSH and AA chickens increased with age. This result was consistent with Mersmann (1998) who found changes in LPL activity with age. Meanwhile, we found that LPL enzyme activity in the thigh was higher than that in breast tissues of either GXSH or AA chickens. LPL enzyme activity in the muscle tissues of these two breeds and age were positively correlated.

When LPL enzyme activity was compared between the two breeds, we found that LPL enzyme activity levels in the breast and thigh tissues of GXSH chickens on days 7, 28, and 56 were significantly lower than those of AA chickens. Therefore, further investigations of LPL genes seem to be necessary to select it as a candidate gene for fatness traits.

IMF content of two chicken breeds

IMF is the material basis of meat tenderness, juiciness, and taste, and there is a significant positive correlation between IMF and meat tenderness, flavor, and juiciness (Lefaucheur et al., 2011). In recent years, research sought IMF contents to reveal the internal molecular mechanisms associated with the regulation IMF deposition has become the hot field of research on nutrition, genetics, etc.

Aging has been reported in animals, and it is often associated with a variety of physiological changes, including those related to body weight, body composition (Thomas et al., 2002), and especially body fat (Elia, 2001). For instance, the percent of body fat in young men is lower than that of middle-aged men (Imbeault et al., 2001). The results of Shan et al. (2006) supported this conclusion in that the capacity of adipose deposition in pigs increased with age. In our study, we found that the IMF contents of different tissues increased with age in the two chicken breeds. The results of the current experiment suggested a correlation between the age and the IMF contents, which is in accordance with previous reports (Cui et al., 2012). In addition, for AA chickens, IMF contents in the two tissues on days 28 and 56 were significantly higher than that of GXSH chickens on days 28 and 56. However, at the marketing age, GXSH chickens (day 120) exhibited higher IMF contents than AA chickens (56). Moreover, the results showed that the fat contents in the muscle tissues of AA chickens were significantly higher than those of GXSH chickens in the same tissues at early development stages. However, AA chickens grew quickly, and fat deposition was small, which harmed the flavor. On the contrary, GXSH chickens grew slowly, and the time for fat deposition was longer than in AA chickens. GXSH chickens achieved the best flavor taste by the marketing time, and the results of this study indicated that the breed was also a key factor that affected IMF contents.

Relationship between *LPL* mRNA expression levels and IMF contents

Goldberg (1996) indicated that LPL is a major enzyme that plays an important role in lipid metabolism by being responsible for the hydrolysis of TG molecules that are present in circulating lipoproteins, and it also hydrolyzes TG to produce free fatty acids that are assimilated by muscle and adipose tissue. The results of Hocquette et al. (1998) supported the hypothesis that LPL can increase fat storage or provide energy storage in the form of fatty acids for muscle growth. Ren et al. (2002) reported that higher *LPL* gene expression in fat depots was correlated with the higher fat content in dairy cattle. For 7- to 28-week-old pigs,

researchers observed significant negative correlations between *LPL* gene expression levels and adipose deposition (Shan et al., 2006). It has been reported that there were no obvious relationships between *LPL* gene expression in muscle tissues and the accumulation of IMF in Xinjiang Merino sheep. However, for 2- to 60-day-old Kazak sheep, there was a negative correlation with *LPL* expression levels and IMF contents during developmental stages (Qiao et al., 2008). Based on the report described above, we can see that the relationship between *LPL* expression levels and fat deposition can be affected by the animal species and animal age. In the present study, we found significant positive correlations between *LPL* gene expression levels and fat contents in the breast and thigh tissues of both GXSH ($r = 0.712$, $P = 0.001$; $r = 0.792$, $P < 0.001$, respectively) and AA ($r = 0.644$, $P < 0.001$; $r = 0.545$, $P < 0.001$, respectively) chickens.

Relationship between LPL enzyme activities and IMF content

Previous studies suggested that LPL may be regulated by different mechanisms in different animal species (Faulconnier et al., 2001; Bonnet et al., 1998, 2000, 2004; Han et al., 2008). These results indicated that LPL may be regulated at a post-transcriptional level by modulating specific activity and protein content or at a pre-translational level by modulating different mRNA levels. In the present study, we found a significant positive correlation between LPL enzyme activities and fat contents in the breast and thigh tissues of both GXSH and AA chickens. The results suggested that LPL may play an active role in the regulation of lipid metabolism in the tissues of GXSH chickens. Therefore, the results might provide a certain theoretical basis for further studies of the molecular mechanism that underlies IMF deposition in chickens.

LPL may be one of the most important genes that influence meat quality, particularly the IMF contents of GXSH chickens. These results may provide valuable information to aid in our understanding of the meat quality differences between GXSH and AA chickens, and it could form a basis for IMF manipulation for the improvement of meat quality.

Conflicts of interest

The authors declare no conflict of interest.

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REFERENCES

André JM, Guy G, Gontier-Latonnelle K, Bernadet MD, et al. (2007). Influence of lipoprotein-lipase activity on plasma triacylglycerol concentration and lipid storage in three genotypes of ducks. *Comp. Biochem. Physiol. A Mol. Integr. Physiol.* 148: 899-902. http://dx.doi.org/10.1016/j.cbpa.2007.09.006

AOAC (1990). Official methods of analysis. 15th edn. Association of Official Analytical Chemists, Arlington, VA, USA.

Bonnet M, Faulconnier Y, Fléchet J, Hocquette JF, et al. (1998). Messenger RNAs encoding lipoprotein lipase, fatty acid synthase and hormone-sensitive lipase in the adipose tissue of underfed-refed ewes and cows. *Reprod. Nutr. Dev.* 38: 297-307. http://dx.doi.org/10.1051/rnd:19980310

Bonnet M, Leroux C, Faulconnier Y, Hocquette JF, et al. (2000). Lipoprotein lipase activity and mRNA are up-regulated by refeeding in adipose tissue and cardiac muscle of sheep. *J. Nutr.* 130: 749-756.

Bonnet M, Faulconnier Y, Hocquette JF, Bocquier F, et al. (2004). Nutritional status induces divergent variations of GLUT4 protein content, but not lipoprotein lipase activity, between adipose tissues and muscles in adult cattle. *Br. J. Nutr.* 92: 617-625. http://dx.doi.org/10.1079/BJN20041240

Broad TE, Burkin DJ, Cambridge LM, Lewis PE, et al. (1995). Four human chromosome 3q and four human chromosome 21 loci map onto sheep chromosome 1q. *Mamm. Genome* 6: 202-205. http://dx.doi.org/10.1007/BF00293014

Chambaz A, Scheeder MR, Kreuzer M and Dufey PA (2003). Meat quality of Angus, Simmental, Charolais and Limousin steers compared at the same intramuscular fat content. *Meat Sci.* 63: 491-500. http://dx.doi.org/10.1016/S0309-1740(02)00109-2

Cui HX, Zheng MQ, Liu RR, Zhao GP, et al. (2012). Liver dominant expression of fatty acid synthase (FAS) gene in two chicken breeds during intramuscular-fat development. *Mol. Biol. Rep.* 39: 3479-3484. http://dx.doi.org/10.1007/s11033-011-1120-8

Elia M (2001). Obesity in the elderly. *Obes. Res.* 9 (Suppl 4): 244S-248S. http://dx.doi.org/10.1038/oby.2001.126

Faulconnier Y, Bonnet M, Bocquier F, Leroux C, et al. (2001). Effects of photoperiod and feeding level on adipose tissue and muscle lipoprotein lipase activity and mRNA level in dry non-pregnant sheep. *Br. J. Nutr.* 85: 299-306. http://dx.doi.org/10.1079/BJN2000275

Gao SZ and Zhao SM (2009). Physiology, affecting factors and strategies for control of pig meat intramuscular fat. *Recent Pat. Food Nutr. Agric.* 1: 59-74. http://dx.doi.org/10.2174/2212798410901010059

Goldberg IJ (1996). Lipoprotein lipase and lipolysis: central roles in lipoprotein metabolism and atherogenesis. *J. Lipid Res.* 37: 693-707.

Guo J, Shan T, Wu T, Zhu LN, et al. (2011). Comparisons of different muscle metabolic enzymes and muscle fiber types in Jinhua and Landrace pigs. *J. Anim. Sci.* 89: 185-191. http://dx.doi.org/10.2527/jas.2010-2983

Han CC, Wang JW, Xu HY, Li L, et al. (2008). Effect of overfeeding on plasma parameters and mRNA expression of genes associated with hepatic lipogenesis in geese. *Asian-australas. J. Anim. Sci.* 21: 590-595. http://dx.doi.org/10.5713/ajas.2008.70472

Harbitz I, Kristensen T, Kran S and Davies W (1992). Isolation and sequencing of porcine lipoprotein lipase cDNA and its use in multiallelic restriction fragment length polymorphism detection. *Anim. Genet.* 23: 517-522. http://dx.doi.org/10.1111/j.1365-2052.1992.tb00170.x

Hocquette JF, Graulet B and Olivecrona T (1998). Lipoprotein lipase activity and mRNA levels in bovine tissues. *Comp. Biochem. Physiol. B Biochem. Mol. Biol.* 121: 201-212. http://dx.doi.org/10.1016/S0305-0491(98)10090-1

Hu H, Wang J, Zhu R, Guo J, et al. (2008). Effect of myosin heavy chain composition of muscles on meat quality in Laiwu pigs and Duroc. *Sci. China C Life Sci.* 51: 127-132. http://dx.doi.org/10.1007/s11427-008-0016-x

Imbeault P, Vidal H, Tremblay A, Vega N, et al. (2001). Age-related differences in messenger ribonucleic acid expression of key proteins involved in adipose cell differentiation and metabolism. *J. Clin. Endocrinol. Metab.* 86: 828-833.

Lefaucheur L, Lebret B, Ecolan P, Louveau I, et al. (2011). Muscle characteristics and meat quality traits are affected by divergent selection on residual feed intake in pigs. *J. Anim. Sci.* 89: 996-1010. http://dx.doi.org/10.2527/jas.2010-3493

Maingrette F and Renier G (2003). Leptin increases lipoprotein lipase secretion by macrophages: involvement of oxidative stress and protein kinase C. *Diabetes* 52: 2121-2128. http://dx.doi.org/10.2337/diabetes.52.8.2121

Merkel M, Eckel RH and Goldberg IJ (2002). Lipoprotein lipase: genetics, lipid uptake, and regulation. *J. Lipid Res.* 43: 1997-2006. http://dx.doi.org/10.1194/jlr.R200015-JLR200

Mersmann HJ (1998). Lipoprotein and hormone-sensitive lipases in porcine adipose tissue. *J. Anim. Sci.* 76: 1396-1404.

Mooradian AD and Albert SG (1999). The age-related changes in lipogenic enzymes: the role of dietary factors and thyroid hormone responsiveness. *Mech. Ageing Dev.* 108: 139-149. http://dx.doi.org/10.1016/S0047-6374(99)00007-X

Moynihan KA and Imai S (2006). *Sirt1* as a key regulator orchestrating the response to caloric restriction. *Drug Discov. Today Dis. Mech.* 3: 11-17. http://dx.doi.org/10.1016/j.ddmec.2006.02.005

Nechtelberger D, Pires V, Söolknet J, Stur I, et al. (2001). Intramuscular fat content and genetic variants at fatty acid-binding protein loci in Austrian pigs. *J. Anim. Sci.* 79: 2798-2804.

Qiao Y, Huang ZG, Li QF, Liu ZS, et al. (2008). Developmental changes of the LPL mRNA expression and its effect on IMF content in sheep muscle. *Agric. Sci. China* 7: 104-111. http://dx.doi.org/10.1016/S1671-2927(08)60028-0

Ren MQ, Wegner J, Bellmann O, Brockmann GA, et al. (2002). Comparing mRNA levels of genes encoding leptin, leptin receptor, and lipoprotein lipase between dairy and beef cattle. *Domest. Anim. Endocrinol.* 23: 371-381. http://dx.doi.org/10.1016/S0739-7240(02)00179-0

Sato K, Akiba Y, Chida Y and Takahashi K (1999). Lipoprotein hydrolysis and fat accumulation in chicken adipose tissues are reduced by chronic administration of lipoprotein lipase monoclonal antibodies. *Poult. Sci.* 78: 1286-1291. http://dx.doi.org/10.1093/ps/78.9.1286

Shan TZ, Wang YZ, Liu YJ, Liu JX, et al. (2006). Developmental expression of the lipoprotein lipase gene in porcine subcutaneous adipose tissue. *J. Anim. Feed Sci.* 15: 621-629.

Spurlock ME, Ji SQ, Godat RL, Kuske JL, et al. (2001). Changes in the expression of uncoupling proteins and lipases in porcine adipose tissue and skeletal muscle during feed deprivation*(1). *J. Nutr. Biochem.* 12: 81-87. http://dx.doi.org/10.1016/S0955-2863(00)00128-5

Takahashi Y and Ide T (1999). Effect of dietary fats differing in degree of unsaturation on gene expression in rat adipose tissue. *Ann. Nutr. Metab.* 43: 86-97. http://dx.doi.org/10.1159/000012772

Thomas MA, Rice HB, Weinstock D and Corwin RL (2002). Effects of aging on food intake and body composition in rats. *Physiol. Behav.* 76: 487-500. http://dx.doi.org/10.1016/S0031-9384(02)00800-4

van der Vusse GJ, Glatz JF, Stam HC and Reneman RS (1992). Fatty acid homeostasis in the normoxic and ischemic heart. *Physiol. Rev.* 72: 881-940.

Wion KL, Kirchgessner TG, Lusis AJ, Schotz MC, et al. (1987). Human lipoprotein lipase complementary DNA sequence. *Science* 235: 1638-1641. http://dx.doi.org/10.1126/science.3823907

Zhang XD, Li QH, Lou LF, Liu J, et al. (2015). High-resolution melting curve analysis of the ADSL and LPL genes and their correlation with meat quality and blood parameters in chickens. *Genet. Mol. Res.* 14: 2031-2040. http://dx.doi.org/10.4238/2015.March.20.13