

Age-related expression profile of the *SLC27A1* gene in chicken tissues

Yan Wang · Qing Zhu · Xiao-Ling Zhao ·
Yong-Gang Yao · Yi-Ping Liu

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Abstract The solute carrier family 27 (SLC27, also known as fatty acid transport proteins [FATPs]) plays important biological roles in cells. However, there is no report about the expression profile of SLC27 member in chicken. In this study, we quantified the expression of *SLC27A1* (*FATP1*) mRNA in a mountainous black-boned chicken breed (MB) and a commercial meat type chicken breed (S01), to discern the tissue and age-related specific expression pattern and their potential involvement in fat deposition and muscle fatty acid metabolism. Real-time quantitative PCR assays were developed for accurate measurement of *SLC27A1* mRNA levels in different tissues from chicken with different ages (0–12 weeks). Expression of *SLC27A1* mRNA was detected in all tissues examined. There was a significantly age-related change of the *SLC27A1* mRNAs in heart, breast muscle (BMW), leg muscle (LMW), liver, and abdominal fat (AF) tissues ($P < 0.05$). The breast muscle and leg muscle tissues had the highest expression of *SLC27A1* mRNA than the other tissues from the same individual at 0, 2 and 4 weeks. The overall *SLC27A1* mRNA level exhibited a “rise-decline” developmental change in all tissues except for breast muscle, subcutaneous fat, and brain. The S01 chicken had a higher expression of the *SLC27A1* mRNA in breast muscle, subcutaneous fat, and heart tissues than the MB chicken.

Our results showed that the expression of *SLC27A1* mRNA in chicken tissues exhibits specific developmental changes and age-related patterns.

Keywords Chicken · Real-time PCR · *SLC27A1* · Age · Breed · Tissue

Introduction

Long chain fatty acids (LCFAs) act as the main energy substrate for many tissues; approximately 70% of the energy supply in heart is from LCFA oxidation [1]. A protein-mediated uptake and transport of LCFAs has been demonstrated in leg muscle [2], liver [3], adipose tissue [4], and heart [5, 6]. The most prominent and best characterized membrane proteins that increase the uptake of LCFAs are FAT/CD36 [7, 8], fatty acid transport proteins (FATPs)/solute carrier family 27 (SLC27) [4, 9, 10], and plasma membrane associated fatty acid binding protein (FABPpm) [11–13].

The SLC27 belongs to a family containing six related members in human and mice, and have different tissue expression patterns [5]. *SLC27A1* is a 71 kDa plasma membrane protein expressed in adipose tissue, heart and skeletal muscle [4, 14, 15]. Previous biochemical analysis for *SLC27A1* from COS cell extracts showed that this protein exhibits long chain acyl-CoA synthetase activity and may be linked to fatty acid transport [16]. Another member, *SLC27A4*, is often related to *SLC27A1* and expresses in a variety of tissues including adipose tissue, brain, liver, skin, and heart in human and mice [5, 17–19]. *SLC27A4* and other SLC27 family proteins carry an acyl-CoA synthetase activity [16, 20], which has led to substantial speculation about their roles in fatty acid uptake.

Y. Wang · Q. Zhu · X.-L. Zhao · Y.-P. Liu (✉)
College of Animal Science and Technology, Sichuan
Agricultural University, Ya'an, Sichuan 625014, China
e-mail: liuyyp578@yahoo.com

Y.-G. Yao
Key Laboratory of Animal Models and Human Disease
Mechanisms of the Chinese Academy of Sciences and Yunnan
Province, Kunming Institute of Zoology, Kunming,
Yunnan 650223, China

However, the other members of SLC27 family, including SLC27A2, SLC27A3, SLC27A5 and SLC27A6, are more distally related to SLC27A1.

Previous studies have demonstrated that the function of SLC27A1 may be involved in fatty acid uptake in adipose tissue and muscle [18]. For example, Maeda et al. [21] reported that mice lacking adiponectin exhibited severe insulin resistance and had low levels of SLC27A1 in skeletal muscle but not in white adipose tissue and liver. In SLC27A1 knockout mice, incorporation of lipid-derived molecules into adipose and muscle tissues was decreased after a high fat bolus or lipid infusion, whereas incorporation into liver and heart was increased [2]. All these data indicated that the depletion of the SLC27A1 led to a redistribution of postprandial fatty acid uptake and triglyceride deposition in vivo.

Compared with these studies of the *SLC27A1* gene in mouse and human, very limited studies have been performed in chicken to evaluate the expression of this gene in muscle and fat tissues. In a recent study, we identified ten single nucleotide polymorphisms in chicken *SLC27A1* gene in commercial pure lines of the meat-type quality chicken, and discerned an association between the haplotypes of the *SLC27A1* gene and some carcass traits [22]. The lack of expression data of the *SLC27A1* gene in chicken makes it difficult to verify the potential role of SLC27A1 in controlling fatty acid uptake and carcass traits. In this study, we aimed to (1) develop a convenient approach to quantify the abundance of *SLC27A1* transcripts in chicken tissues and (2) to determine potential age-related expression pattern of these genes, particularly in muscle and fat tissues.

Materials and methods

Animals

Sixty-six male chickens at different ages (0, 2, 4, 6, 8, 10, and 12 week) from two breeds/populations, mountainous black-boned chicken (MB) and a commercial chicken (S01) were used in this study. MB chicken is a famous native breed of Sichuan Province, with spotty feathers, black or yellow skin and a favorable meat quality. MB chickens

were raised on the experimental farm for poultry breeding in Sichuan Agricultural University. The pure line S01 was developed by Sichuan Dahan Poultry Breeding Company using local breeds from Sichuan and Guangdong Provinces of China. S01 chicken has yellow partridge plumage with blue shanks and white skin and produces high quality meat. These animals were slaughtered and different tissues (including heart, liver, brain, leg muscle, breast muscle, abdominal fat and subcutaneous fat) were collected. Tissue samples were snap-frozen in liquid nitrogen and then stored at -80°C for total RNA extraction.

RNA isolation and cDNA synthesis

Total RNA was isolated from the heart, liver, brain, leg muscle, breast muscle, abdominal fat, and subcutaneous fat tissues by using the TRIzol reagent (Invitrogen). The quality of RNA was determined by the $A_{260/280}$ absorbance ratio (1.6–1.8) and the integrity of the 18 and 28 S rRNA bands on 1% formaldehyde agarose gel. Isolated RNA samples were treated with 8 μl DNase (Fermentas) at 37°C for 20 min and stored at -80°C .

The cDNA was synthesized using the ImProm-II Reverse Transcription System (TakaRa Biotechnology Co. Ltd., Dalian, China) according to the manufacture's instruction. The reaction was performed in a volume of 10 μl containing $5 \times$ PrimerScript Buffer, 10 mM of each dNTPs, 40 U/ μl RNase Inhibitor, 2.5 μM oligo-dT Primer. The reverse transcription was maintained at 30°C for 10 min, then 45°C for 25 min, and ended with an incubation at 95°C for 5 min. The cDNA product was stored at -20°C .

Real-time PCR assay for *SLC27A1* mRNA expression

Chicken mRNA sequences of the β -actin gene (GenBank accession number AF047874) and *SLC27A1* (accession no. NM001039602) were retrieved from GenBank. Primers were designed by using Primer 3 (http://www.Genome.wi.mit.edu/genome_software/other/primer3.html) (Table 1) and were synthesized by TaKaRa Biotechnology Co., Ltd

Table 1 Primer pairs for quantification of *SLC27A1* and β -actin mRNAs

Primer name	Primer sequence (5' → 3')	Annealing temperature ($^{\circ}\text{C}$)	Product length (bp)
SLC27A1-F	TACGGAGCCACCGAGTGCAACT	61	160
SLC27A1-R	CGCACAGCCCTCTGGAATCACG		
β -actin-F	GAGAAATTGTGCGTGACATCA	61	152
β -actin-R	CCTGAACCTCTCATTGCCA		

(Dalian, China). The length of the amplified fragment was between 60 bp to 150 bp to achieve the most efficient amplification [23].

PCR amplification was performed in a final volume of 10 μ l containing 5 μ l Master mix (Beijing TIAN WEI Biology Technique Corporation, Beijing, China), 0.3 μ l of each primer (10 μ M), and 3.6 μ l ddH₂O with the following amplification conditions: one cycle of 3 min at 94°C; 35 cycles of 30 s at 94°C, 40 s at the respective annealing temperature of each primer pair (Table 1), 45 s at 72°C; and a final extension for 8 min at 72°C. The authenticity of the amplified fragment was verified by sequencing.

The expression levels of chicken *SLC27A1* gene were detected by using the SYBR Green I assay on an IQ5 real-time PCR (RT-PCR) thermal cycle instrument (Bio-Rad, German). RT-PCR reaction was performed in a volume of 12.5 μ l containing 10 mM Tris-HCL (pH 8.3), 50 mM KCL, 3.5 mM MgCL₂, 50 U/ml Takara ExTaq™ R-PCR (TakaRa Biotechnology Co. Ltd., Dalian, China), 0.2 μ M of each specific primer and 0.25 μ l/300 diluted SYBR Green I (TakaRa Biotechnology Co. Ltd., Dalian, China). The cycling condition consisted of an initial denaturation cycle for 3 min at 95°C; 35 cycles of 30 s at 94°C, 40 s at 61°C, and a final extension step at 72°C for 15 s. In order to verify no non-specific amplification, following the completion of the RT-PCR, melting curve analysis was performed. The melting protocol consisted of heating from 55 to 95°C at a rate of 0.5°C per step, and each step was held for 1 s for data acquisition. Standards curves were generated using 10⁻³–10⁻¹⁰ dilution series template of PCR product for the *SLC27A1* and *β -actin* genes.

Statistical analysis

Gene expression levels were quantified relatively to the expression of the *β -actin* according to the formula as followed [24]:

$$\text{Rel. Quantity} = \frac{(1 + \text{Eff})_{GOI}^{(Ct_{Control} - Ct_{Sample})}}{(1 + \text{Eff})_{NORM}^{(Ct_{Control} - Ct_{Sample})}}$$

Where Rel. Quantity is the relative quantity of the *SLC27A1* in a sample compared with that in the control, GOI is the gene of interest, NORM is the reference gene and Eff is the efficiency of the quantitative RT-PCR. The cDNA dilution curves were generated and used to calculate the individual real-time PCR efficiencies ($E = 10^{[-1/\text{slope}]}$). Expression data were described as means \pm SD and were analyzed using the SAS 6.12 for Windows Software (SAS Institute Inc., Cary, NC). Gene expression levels between different tissues from the age-related samples of the same breed were analyzed by one-way ANOVA. We also

quantified the expression levels of the *SLC27A1* gene between the MB and S01 breeds using the independent sample *t*-test. A *P* value < 0.05 was considered as statistically significant.

Results

Standard curves and melting curves of the *SLC27A1* and *β -actin* genes

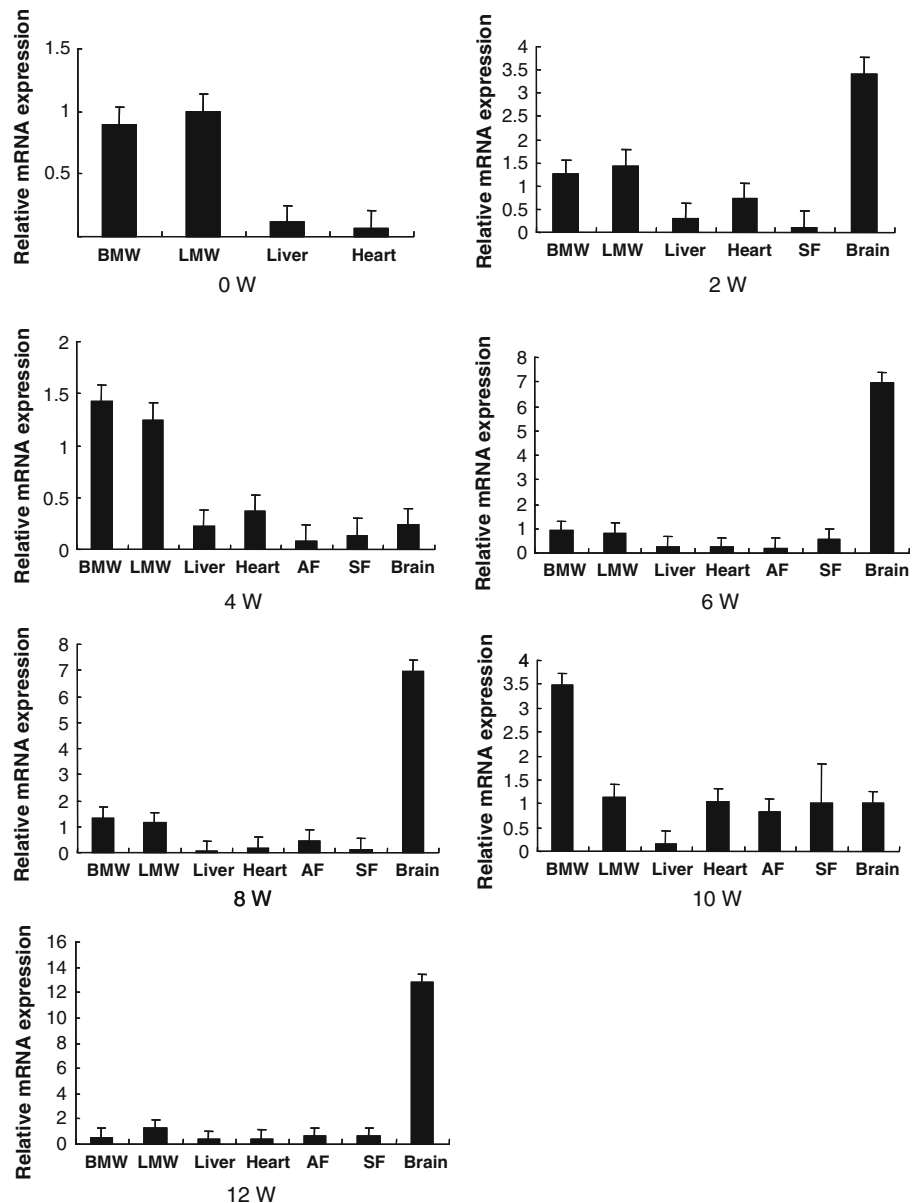
Serial dilutions (10⁻³–10⁻¹⁰) of the PCR products for the *SLC27A1* and *β -actin* genes in chicken liver tissue were tested by the RT-PCR, respectively. The crossing point, where the sample's fluorescence curve turns sharply upward, indicating exponential amplification, were automatically determined by the RT-PCR software as 10.05–26.65, and the range of the Ct values for the *β -actin* gene was 9.70–28.83. Plotting the obtained Ct values relative to the serial dilutions of the *SLC27A1* and *β -actin* resulted in a linear correlation ($Y = -3.333X - 1.700$ for *SLC27A1* and $Y = -3.341X + 5.083$ for *β -actin*) with square regression coefficient of 0.995 and 0.992, respectively, suggesting that quantification of the target DNA was possible. The average slopes of for the *SLC27A1* and *β -actin* genes were -3.33 and -3.34, respectively. According to the formula $\log E = \text{slope}^{-1}$, the current PCR reaction efficiencies are above 99.6% for the *SLC27A1* gene and 99.2% for the *β -actin* gene.

Expression of the *SLC27A1* mRNA in different chicken tissues

The expression of the *SLC27A1* mRNA was detected in all seven chicken tissues analyzed in this study. Relative to the *β -actin* gene, the expression levels of the *SLC27A1* mRNA varied considerably in different tissues (Fig. 1). Compared with the expression pattern of the *SLC27A1* mRNA in other tissues, the *SLC27A1* transcript had a relatively higher expression in leg muscle, brain, and breast muscle tissues. Specifically, leg muscle and breast muscle had a high expression of *SLC27A1* mRNA at an age of 4 week or younger, whereas brain tissue had a significantly high expression of this gene after 6 week ($P < 0.05$). The heart tissue, despite that its energy supply is heavily dependent on LCFA oxidation [1], had a consistent lower level of *SLC27A1* mRNA. Similar pattern was also observed for liver tissue, abdominal fat and subcutaneous fat (Fig. 1).

The developmental changes of the *SLC27A1* mRNA expression in chickens with different ages were also analyzed. As shown in Fig. 2, the *SLC27A1* mRNA expression

Fig. 1 The relative expression of the *SLC27A1* gene in different chicken tissues. For each growth point, we used the Ct value of leg muscle as the control to calculate the expression values of the *SLC27A1* mRNA in other tissues. *LMW* leg muscle, *BMW* breast muscle, *SF* Subcutaneous fat, *AF* Abdominal fat. For each growth point, at least six male chickens were analyzed



level in breast muscle, leg muscle, abdominal fat, heart, brain and liver varied significantly at different age ($P < 0.05$). The *SLC27A1* mRNA in breast muscle had the highest expression at 12 week and the lowest expression at 6 week, and exhibited a “decline-rise” developmental change. However, the leg muscle had the highest expression the *SLC27A1* mRNA at 2 week and the lowest expression at 10 week (Fig. 2a). Both liver and heart tissues had the highest expression of the *SLC27A1* gene at 2 week. In brain tissue, the highest expression of the *SLC27A1* gene was at 12 week (Fig. 2b). In abdominal fat, the *SLC27A1* mRNA expression levels were significantly different at various weeks of age ($P < 0.05$) and exhibited a “rise-decline-rise” developmental change. In contrast, the *SLC27A1* expression level in subcutaneous fat

exhibits a “decline-rise-decline-rise” developmental change (Fig. 2c).

Comparison of the *SLC27A1* gene expression pattern between MB and S01 chickens

To further characterize whether the expression of the *SLC27A1* gene had a breed specific feature, we analyzed the expression level of this gene in two chicken breeds. Table 2 showed that there was no significant difference of the *SLC27A1* mRNA between S01 and MB chickens ($P > 0.05$). However, we found that the *SLC27A1* mRNA levels in breast muscle, subcutaneous fat, and heart tissues were slightly higher in S01 than those in MB chicken.

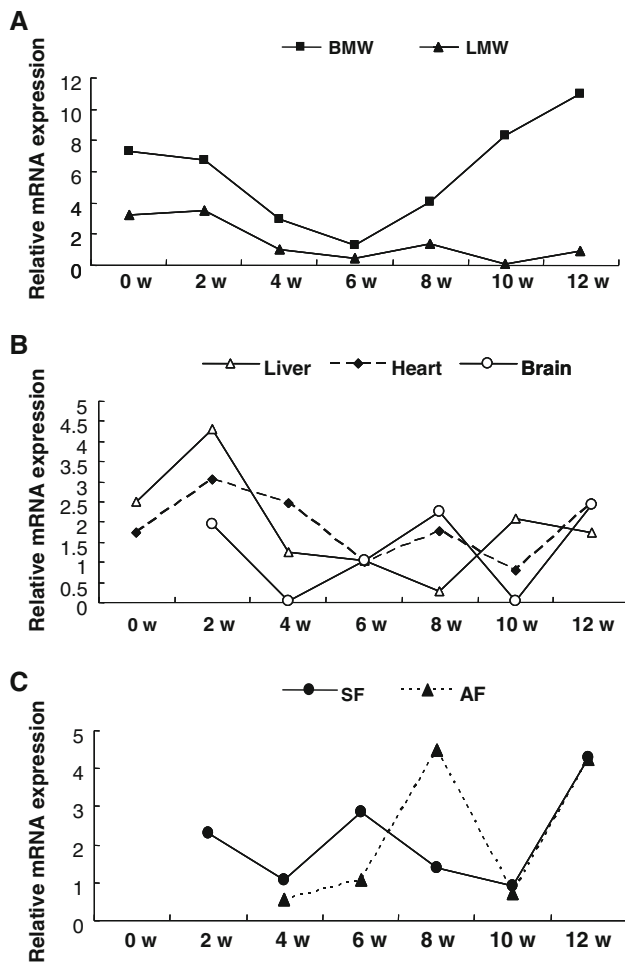


Fig. 2 The relative expression of the *SLC27A1* gene during chicken development. For each tissue, we used the Ct value at 12 week as the control to calculate the values of the *SLC27A1* gene at different growth points. *LMW* leg muscle, *BMW* breast muscle, *SF* Subcutaneous fat, *AF* Abdominal fat

Discussion

The closely related members of the *SLC27* family exhibit both fatty acid transport and acyl CoA synthetase activities [18, 25]. To date, most of studies on *SLC27* have been carried out on mammalian species, such as human and mouse. Little is known about *SLC27* expression in chicken tissues. Because the expression levels of *SLC27A1* gene are relatively low in certain chicken tissues, using the highly

sensitive real-time PCR method enables us to accurately detect the lower level of the transcript, particularly when very small amounts of tissues are available. In this study, we established a quantitative RT-PCR method to evaluate the *SLC27A1* mRNA expression in different chicken tissues at different ages. We found that the *SLC27A1* gene had an expression in tissues with active fatty acid metabolism, such as heart, leg muscle, breast muscle, liver, which was in accordance with the recent report on rat [26].

Several studies have shown the presence of the *SLC27A1* protein in skeletal muscle [4, 14, 15, 27]. In rat, higher level of *SLC27A1* has been reported in the soleus muscle (oxidized type) than in the gastrocnemius muscle (glycolysed type) [2, 28]. Song et al. [28] also reported that the *SLC27A1* mRNA expression level in leg muscle was much higher than that in chest muscle. In our study, we found that the expression levels of the *SLC27A1* mRNAs in chicken breast muscle and leg muscle were relatively higher than those of other tissues at younger age. Brain tissue had significantly higher level of *SLC27A1* gene expression at four of the nine growth points that were analyzed in this study. Chicken leg muscle and breast muscle belong to two different types: the former is mainly composed of IIB type myofiber (glycolysed type), where energy came from glycolysis of carbohydrate; the latter is I type (oxidized type), which utilized aerobic oxidation of fatty acids instead [27]. Despite a fact that our result was somewhat inconsistent with that reported by Song et al. [28], we all showed the expression of the *SLC27A1* in muscle tissues, possibly as one of the transporters of long chain fatty acids and played an important role in the fatty acid regulation in muscles. Further studies are needed to clarify the relationship between *SLC27A1* and fatty acid metabolism in muscle.

Different from mammals, fat deposition in birds was reported to depend on exogenous fatty acid transportation much more than de novo synthesis [29–31]. Several studies have indicated that *SLC27A1* directly mediates fatty acid transmembrane transportation and catalyzes the intracellular fatty acids into acyl-CoA, preferentially for intracellular triglyceride synthesis [27, 32]. In the present study, the *SLC27A1* mRNA expression levels in the abdominal fat and subcutaneous fat tissues were relatively lower than those in other tissues (Fig. 1). It remains unclear why the

Table 2 Relative expression of the *SLC27A1* mRNA in chickens from two different breeds at 10 week

Breeds	Sample size	BMW	AF	SF	Liver	Heart
S01	6	1.105 ± 0.221	1.025 ± 0.446	1.221 ± 0.324	1.009 ± 0.546	1.262 ± 0.383
MB	6	1.004 ± 0.192	1.275 ± 0.386	1.038 ± 0.375	1.415 ± 0.473	1.105 ± 0.383

MB Mountainous black-boned chicken, *BMW* breast muscle, *SF* Subcutaneous fat, *AF* Abdominal fat

SLC27A1 mRNA expression had such a pattern in these tissues directly related to fat deposits. Whether there is an inconsistency of chicken *SLC27A1* mRNA expression and protein expression in these two fat tissues needs further investigation.

In order to determine the breed effect on the *SLC27A1* gene expression, we analyzed several tissues from individuals belonging to two different breeds, which exhibited various physiological features and carcass traits. The MB chicken, an indigenous breed of Sichuan Province, has spotty feather and black or yellow skin and a favorable meat quality, but grows slowly. In contrast, the S01 chicken is a foster species established by Sichuan Dahan Poultry Breeding Company; this chicken has favorable meat quality and grows very fast. Although the number of samples in each group was not large, we found some differences in *SLC27A1* mRNA expression between the chickens from the two breeds. The *SLC27A1* mRNA expression levels in breast muscle, subcutaneous fat and heart tissues of S01 chicken are slightly higher than those in MB. Conversely, MB chickens have a higher level of *SLC27A1* mRNA expression in liver and abdominal fat tissues (Table 2). It seems that the different expression levels of *SLC27A1* mRNA may be correlated with the characters of these two breeds. Indeed, we have shown that genetic variants in this gene might affect chicken carcass traits [22]. Further studies of additional breeds are necessary to assess whether changes in *SLC27A1* mRNA in breast muscle, subcutaneous fat, abdominal fat, liver, and heart correlate with the levels of their cognate protein and whether such changes can be predictive or be causally related to physiological status of breeds.

In conclusion, we have developed a highly sensitive RT-PCR method for assessing *SLC27A1* mRNA in different tissues from chickens with different ages. The *SLC27A1* mRNA expression presented a developmental change with increased age. In particular, leg muscle and breast muscle had a higher expression of *SLC27A1* mRNA at age of 4 week or younger, whereas brain tissue had a significantly high expression after 6 week. In addition, we found that *SLC27A1* mRNA levels presented a breed-related expression pattern, with higher expression in breast muscle, subcutaneous fat, and heart tissues of S01 chicken than MB chicken. All these results showed that the expression of *SLC27A1* mRNA in chicken tissues exhibits specific developmental changes and age-related patterns.

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