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Developmental Changes in Myofibers and Expression Profiles of Potential Regulatory Genes in Slow- and Fast- Growing Chickens

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Authors' Contribution
XZ designed the study. ZW, GS, LY and LZ raised the chickens and performed the experiments. QZ, YW, HZ, ZZ and DL helped in experimental work. JL analyzed the data and wrote the article. AMS, ZN and YT helped in preparation of manuscript.

Key words
Broiler, Myofiber development, Myoblast determining factors, Myogenin, Expression abundance

ABSTRACT

There are weight and size differences in skeletal muscles of fast- (FG), and slow- growing (SG) chickens, however, the underlying molecular mechanisms responsible for the differences in post-hatch muscle development are unclear. Here, we report on the identification of several candidate genes that may modulate myofiber growth and thus explain some of the differences in the skeletal muscle phenotype of FG and SG chickens. We collected pectoralis major (PM) and gastrocnemius muscles (GM) on d 1, 7, 28, 49, and 70 post-hatch, and measured the weights of the muscles, diameter and, a density of myofibers, and the expression abundances of *MyoD*, *MyoG*, *IGF-1*, and *Pax7*. The body weight of FG was heavier than SG from d 7 to 70. Their muscle weights and myofiber diameters of FG were also greater than SG ($P < 0.05$). The expression of *MyoG* was affected by chicken population, age, and muscle-type. It was the heaviest in PM for FG on d 28, compared with other combinations. The expression of *Pax7* mRNA paralleled changed in myofiber density, whereas the expression profiles of *MyoG* and *MyoD* were similar to the developmental changes in myofiber diameter. Overall, body weight and muscle expansion of SG chickens were less than FG chickens. *MyoG* was the possible gene controlling myofiber development as its expression abundances were associated with the muscle development profiles in the SG and FG chickens, respectively.

INTRODUCTION

Development of skeletal muscle is an economically-important trait in poultry production. It is well known that the myofiber number in chickens is established before hatching. So, any increase in muscle weight post-hatching depends on the increase in length and diameter of the myofibers (Chen *et al.*, 2007). Myofiber growth is affected by interactions of heredity, age, nutrition, exercise, type of management and environmental conditions (Hu *et al.*, 2013; Michalczuk *et al.*, 2016). In China, local meat-type chickens have a slower growth rate and smaller myofiber diameters than commercial strains (Sheng *et al.*, 2013). Consumer acceptance of meat depends on its quality, which is influenced by a series of factors ranging from the physical and chemical to the histological properties and processing procedure of meat. Tenderness has been noted as the most important factor in consumer perception of quality of meat products. Papa (1988) had demonstrated that myofiber diameters influenced the tenderness of meat products (Papa and Fletcher, 1988). Studies have identified multiple genes that contribute to the growth and development of skeletal muscle fibers in mammals, such as myoblast determining factors (*MyoD*), myogenin (*MyoG*), insulin like growth factor 1 (*IGF-1*), and paired box 7 (*Pax7*) (Grochowska *et al.*, 2017; Stupka *et al.*, 2014; Wang *et al.*, 2015). Their functions in the development of SG and FG chickens' skeletal muscle

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are unclear. This experiment thus focused on the skeletal myofiber characteristics and gene expression profiles of potential regulatory factors in the slow- growing genetic line HS1 (SG) and fast-growing genetic line Cobb (FG). The HS1 is a Chinese dual-purpose chicken line selected for five generations by Sichuan Agricultural University in China. It originated from a local breed that grows slowly during the starter and grower periods and has good meat quality. Cobb is a typical commercial population that was successfully bred in Britain to meet the demands of the fast-growing bird market (Taschetto *et al.*, 2012).

MATERIALS AND METHODS

All procedures for raising and slaughtering chickens were approved by the Institutional Animal Care and Use Committee of Sichuan Agricultural University. The methods were conducted according to approved guidelines.

Sampling

A total of 180 one-day-old males (90 birds from each population) were raised for this study, with three replicate groups (30 chicks per group) in each population. All the chicks were raised in batteries with wire mesh floor from d 1 to d 70.The diets consisted of 21.4% CP and 3,015 Kcal of ME/kg to d 28, followed by 19.9% CP and 3,100 kcal of ME/kg from d 29 to 42, and 18% CP and 3,180 kcal of ME/kg from d 43 to 70. Water and feed were available ad libitum. We weighted the birds at the first day and the end of each week from d 1 to 70, and randomly sampled six birds from each group on d 1, 7, 28, 49, and 70, respectively. The whole right breast muscle (pectoralis major [PM] and minor) and leg muscles (drum and thigh) of each chicken were isolated and weighed. Breast muscle weight (BW) and leg muscle weight (LW) are shown as doubled right breast muscle weight and leg muscle weight, respectively. About two cm^3 of right PM and gastrocnemius muscle (GM) were excised along the muscle fibers and fixed in formalin for paraffin embedding and sectioning. Samples for RNA extractions were collected from the left PM and GM, snap-frozen in liquid nitrogen, and stored at -80°C.

Morphological analysis of skeletal muscles

PM and GM tissues were fixed with 10% neutral-buffered formalin and then dehydrated in a dilution series of ethanol and treated with xylene. Samples were embedded into paraffin blocks, trimmed, and cut at 5 μm thickness using a Microm HM315 (Germany), sections were mounted onto slides. Dewaxed after section flattening, slices were stained with hematoxylin and eosin (Saverino *et al.*, 2014). We observed a section under the microscope with a magnification of 10×20x, randomly selected 5-10 fields on the image, and captured the photomicrographs. The diameter and density of myofibers were measured with Image-Pro Plus 5.0 software (Media Cybernetics Bethesda, MD, USA).

Total RNA extraction and cDNA synthesis

Total RNA was isolated from PM and GM using Trizol (Invitrogen, USA) according to the manufacturer's instructions. The integrity and concentration/quality of RNA were verified by gel electrophoresis and spectrophotometry, respectively. The cDNA was synthesized via reverse transcription with a PrimeScript® RT reagent Kit (TaKaRa Biotech Co., Ltd.).

Real time PCR

A SYBR Prime Script RT-PCR Kit (TaKaRa, Japan) was used for real time PCR. *β-Actin* was the housekeeping gene. The reaction contained 2 μL of cDNA template, 12.5 μL SYBR® Premix Ex Taq™II, 8.5 μL ddH_2O, and 1 μL of each gene-specific primer (Table I). The reaction mixture was predenatured for 2 min at 95 °C, followed by 40 cycles at 95 °C for 5 s, 65 °C for 2 s, and a full extension cycle at 95 °C for 5 s. Reactions were performed in triplicate. Primer sequences are displayed in Table I.

Data analysis

The model for other traits including myofiber diameter and density, breast muscle and leg muscle weights were as follows.

$$Y_{ijk} = \mu + P_i + T_j + A_k + (PT)_{ij} + (PA)_{ik} + (TA)_{jk} + (PTA)_{ijk} + e_{ijk}$$

where Y_{ijk} = the performance of chicken in population i of tissue j on age k, μ = the general mean, P_i = the effect of population i (i = 1 and 2; FG and SG), T_j = the effect of tissue j (j = 1 and 2; breast muscle and leg muscle), A_k = the effect of age k (k = 1, 2, 3, 4, and 5; d1, 7, 28, 49, and 70) ; $(PT)_{ij}$ = the interaction effect of population i × tissue j, $(PA)_{ik}$ = the interaction effect of population i × age k, $(TA)_{jk}$ = the interaction effect of tissue j × age k, $(PTA)_{ijk}$ = the interaction effect of population i × tissue j × age k, e_{ijk}= the random residual effect.

All data were analyzed using the GLM procedure of JMP Pro v.10 (SAS Institute). Tukey's test was used for multiple comparison analysis, and statistical significance was set at $P < 0.05$. Live weights at the end of each week were analyzed via student's t-test.

RESULTS

The body weights of the two populations from d 1 to 70 were shown in Fig 1. Student's t-test results indicated that the body weight of the chickens did not differ between FG and SG at hatch, whereas FG was heavier than SG from d 7 to 70 ($P < 0.05$).

Table I.- Primers used for real time PCR.

Gene[1]	Primer sequence[2] (5′–3′)	Product length (bp)	Annealing temperature (°C)	GenBank accession number
β-Actin	F:TGTGCTGTCCCTGTATGCCTC R: GGAGGGCGTAGCCTTCATAGA	101	60	NM_205518.1
IGF-1	F:GTGGTGCTGAGCTGGTTGATG R: AGCATTCATCCACTATTCCCTTG	126	58	NM_001004384.2
MyoD	F:GCTACTACACGGAATCACCAAATG R: CATGTGGAGTTGTCTGTGGAAATC	112	58	NM_204214.2
MyoG	F:GCGGAGGCTGAAGAAGGTGA R: CGCTCGATGTACTGGATGGC	120	57	NM_204184.1
Pax7	F:CCACGGGAATGCCAACTCT R: ATGGTGGATGGTGGCAAGG	121	57	NM_205065.1

[1] IGF-1, *insulin-like growth factor 1*; MyoD, *myogenic differentiation antigen*; MyoG, *myogenin*; Pax7, *paired-box 7*.
[2] F, forward primer; R, reverse primer.

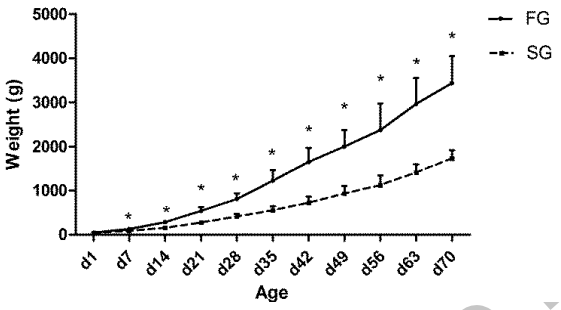

Fig. 1. Body weights of FG and SG from d 7 to 70. Means with "*" differ significantly ($P < 0.05$).

Effects of Interaction of population, age, and tissue on myofiber histological characteristics

Results for the histology analysis were summarized in Table II. The results showed that the effect of age on all traits was significant. The muscle weight of the breast and leg and myofiber diameter of PM and GM for males increased with age, whereas their myofiber density decreased (Table II; $P < 0.001$). Meanwhile, the muscle weight and myofiber diameter of FG were greater than SG ($P < 0.01$). The myofiber diameter of PM was greater than GM ($P < 0.001$).

Effects of Interaction of population with age on muscle fiber characteristics

There was an interaction of population and age on muscle fiber characteristics (Fig. 2). The muscle weight (Fig. 2a) and myofiber diameter (Fig. 2b) of FG and SG were the highest on d 70 in comparison with the other combinations, and the muscle weights of FG were greater than SG from d 28 to 70 ($P < 0.05$) (Fig. 2a). The myofiber diameters of FG were thicker than SG from d 1 to 70 ($P < 0.05$) (Fig. 2b). While, myofiber density (Fig. 2c) decreased from d 1 to 7 and did not significantly differ between the two populations at the same time point ($P > 0.05$).

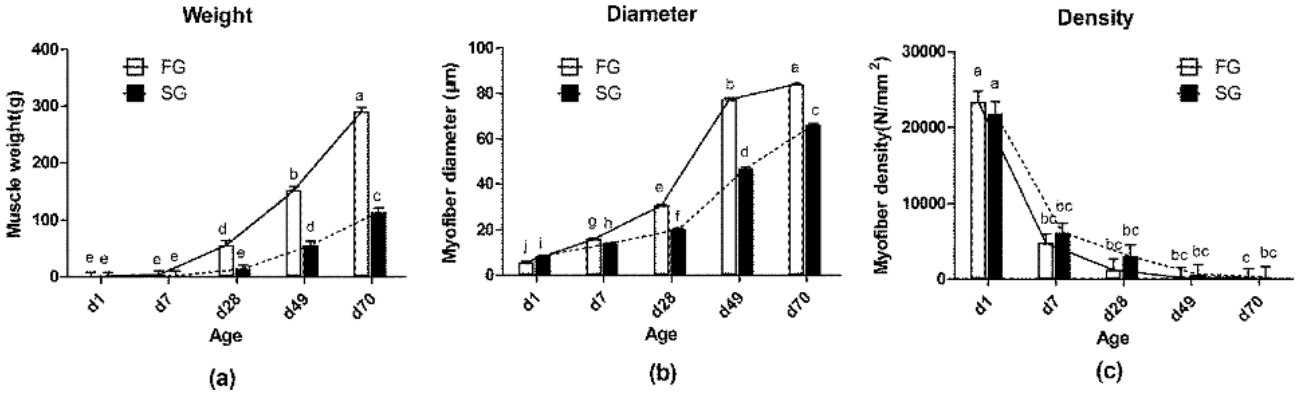

Fig. 2. Myofiber characteristics of populations FG and SG on d 1, 7, 28, 49, and 70. (a) Muscle weight, (b) myofiber diameter, (c) myofiber density. Means without a common lowercase differ significantly ($P < 0.05$).

Table II.- Muscle fiber characteristics of chickens on d 1, 7, 28, 49, and 70.

Effects		n	Diameter[1] (μm)	Density[1] (N/mm²)	Weight[1] (g)
Population	FG	30	37.07[a]	5514.27	98.55[a]
	SG	30	25.39[b]	5618.44	38.17[b]
	SEM		0.85	916.60	16.10
	P-value		< 0.001	0.95	0.01
Age	d1	12	7.08[e]	22695.11[a]	0.57[c]
	d7	12	14.84[d]	5413.36[b]	2.56[c]
	d28	12	26.26[c]	2102.66[bc]	28.96[c]
	d49	12	61.91[b]	403.02[c]	111.79[b]
	d70	12	76.15[a]	225.04[c]	194.14[a]
	SEM		0.65	855.83	0.70
	P-value		< 0.001	< 0.001	< 0.001
Tissue	Pectoralis major muscle	60	27.79[b]	7852.10	58.79
	Gastrocnemius muscle	60	37.05[a]	4800.10	78.64
	SEM		0.89	1044.05	12.03
	P-value		< 0.001	0.15	0.41
			P-value		
Interaction[2]	P×A		< 0.001	< 0.001	< 0.001
	P×T		< 0.001	0.23	0.07
	A×T		< 0.001	< 0.001	< 0.001
	P×A×T		< 0.001	< 0.001	< 0.001

[1]Means in a column within an effect without a common superscript letter differ significantly ($P < 0.05$).
[2]P, A, and T represent effects of population, age, and tissue, respectively.

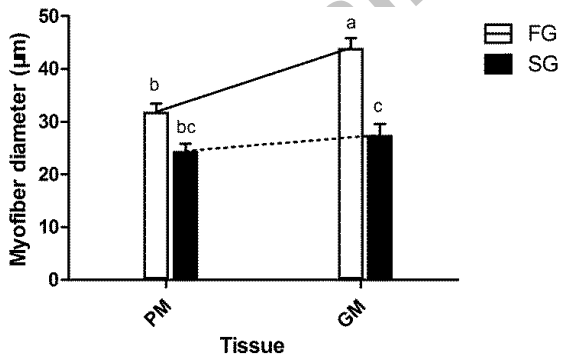

Fig. 3. Myofiber characteristics (myofiber diameter) of PM and GM for populations FG and SG. Means without a common lowercase differ significantly ($P < 0.05$).

Effect of Interaction of population and tissue on muscle fiber diameter

There was an interaction of population and tissue on muscle fiber traits as shown in Figure 3. The GM fiber diameter of FG was thicker than SG, and the fiber diameter in PM was less than GM for FG ($P < 0.05$). No differences were observed between the tissues of SG ($P > 0.05$).

Effects of Interaction of tissue and age on muscle fiber characteristics

Figure 4 shows the interaction of tissue and age on muscle fiber characteristics. No significant differences were observed between BW and LW ($P > 0.05$) (Fig. 4a). The fiber diameter in PM was greater than GM on d 28 ($P < 0.05$) (Fig. 4b). However, the opposite pattern was observed from d 49 to onward. In addition, myofiber density declined with time, and the myofiber density of PM was greater than GM on the first day ($P < 0.05$) (Fig. 4c).

Effects of Interaction of population, age, and tissue on myofiber characteristics

The three-way interaction is displayed in Figure 5. From d 28 to 70, muscle weights of FG were heavier than SG, and the leg of SG was heavier than the breast of SG on

d 70 ($P < 0.05$) (Fig. 5a). The PM fibers of FG were thicker

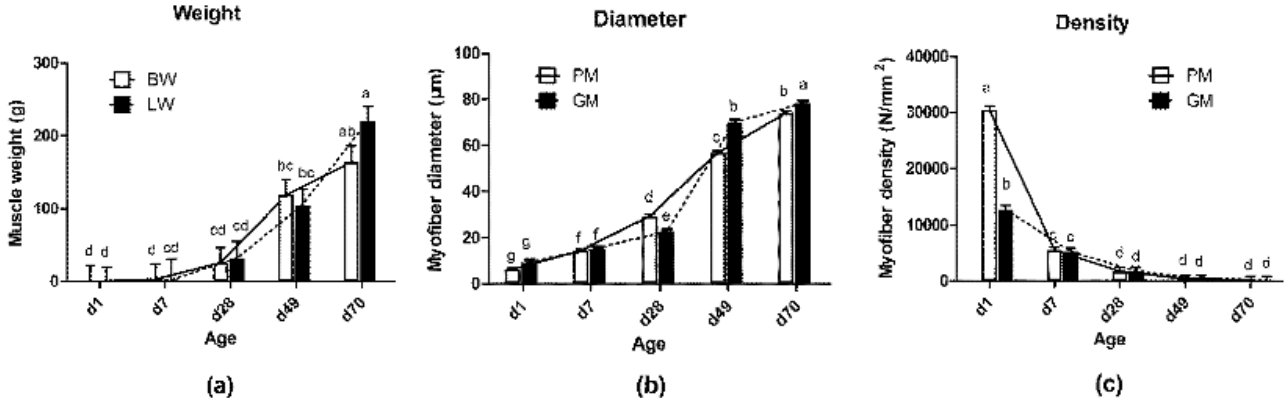

Fig. 4. Myofiber characteristics of PM and GM on d 1, 7, 28, 49, and 70. (a) Muscle weight, (b) myofiber diameter, (c) myofiber density. BW = doubled pectoralis major and minor muscle weights, LW = doubled drum and thigh muscle weights. Means without a common lowercase letter differ significantly ($P < 0.05$).

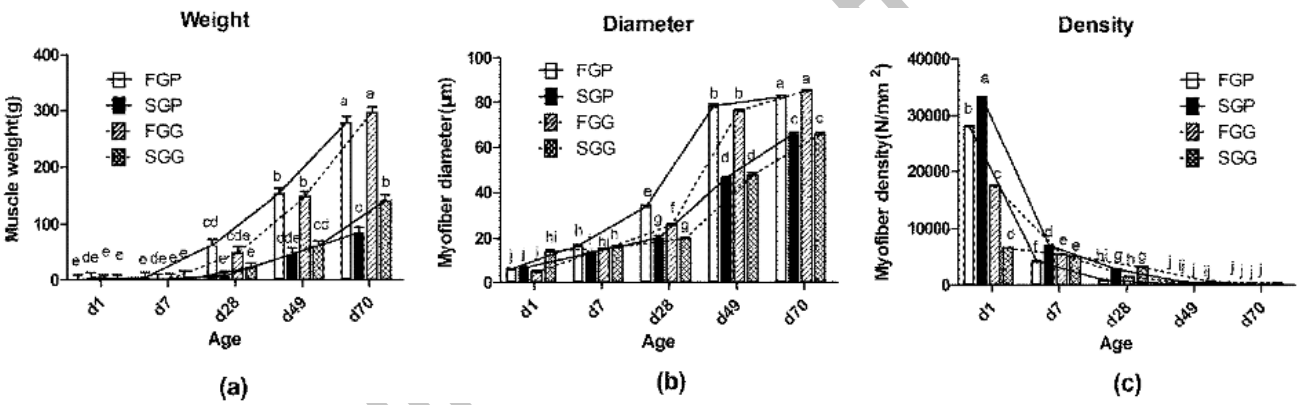

Fig. 5. Myofiber characteristics of two populations for PM and GM on d 1, 7, 28, 49, and 70. (a) Muscle weight, (b) myofiber diameter, (c) myofiber density, FGB = breast of FG, SGB = breast of SG, FGL = leg of FG, SGL = leg of SG, FGP = PM of FG, FGG = GM of FG, SGP = PM of SG, SGG = GM of SG. Means without a common lowercase letter differ significantly ($P < 0.05$).

than SG from d 7, and the GM fiber diameters of FG were greater than SG from d 28 ($P < 0.05$) (Fig. 5b). In the first week, the GM fiber diameter was greater than PM's in SG ($P < 0.05$) (Fig. 5b). On d 28, the PM fiber was thicker than GM's in FG ($P < 0.05$) (Fig. 5b). In general, the myofiber diameters of FG were greater than SG for all growth points ($P < 0.05$) (Fig. 5b). The myofiber densities for both SG and FG were the greatest on the first day compared with other growth points. From d 1 to 28, the PM fiber density of SG was greater than FG. The fiber density in GM of FG was greater than GM of SG on day 1, but fiber density in GM of SG exceeded that in GM of FG on d 28 ($P < 0.05$) (Fig. 5c). The fiber density in PM was greater than GM for FG during the first week. The fiber density of PM was greater than GM for FG on d 1, and its GM fiber density exceeded that of PM on d 7 ($P < 0.05$) (Fig. 5c).

In general, muscle weight and myofiber diameter in chickens were increased with time while myofiber density was decreased with time. Moreover, the muscle weights and myofiber diameters in fast-growing Cobbs were greater than slow-growing chicken line HS1 from d 28 ($P < 0.05$).

Effects of population, age, and tissue on mRNA

In Table III we summarized the expression of genes *MyoD*, *MyoG*, *IGF-1*, and *Pax7*, including the main effects of population, age, tissue, and their interactions. The effect of age was significant in this study. The expression profiles of *MyoG* and *IGF-1* increased initially, then decreased, and reached its peak on d 7. *Pax7* expression decreased with time ($P < 0.05$). In addition, *MyoG* and *Pax7* mRNA abundances were greater in the PM than the GM ($P <$

0.05).

Table III.- Effects of population, age, and tissue and their interactions on gene expression abundances in skeletal muscles.

Effects		n	Relative difference in mRNA			
			MyoD[1]	***MyoG***[1]	***IGF-1***[1]	***Pax7***[1]
Population	FG	30	1.02	1.07	0.79	1.07
	SG	30	0.95	0.82	0.73	1.17
	SEM		0.11	0.09	0.06	0.19
	P-value		0.74	0.15	0.64	0.80
Age	d1	12	1.31	0.52^{b}	0.71^{b}	2.07^{a}
	d7	12	0.80	1.27^{a}	1.24^{a}	0.94^{ab}
	d28	12	1.39	1.10^{ab}	0.68^{b}	1.59^{ab}
	d49	12	0.63	1.36^{a}	0.71^{b}	0.57^{ab}
	d70	12	0.69	0.50^{b}	0.54^{b}	0.28^{b}
	SEM		0.22	0.16	0.12	0.38
	P-value		0.042	< 0.001	0.005	0.01
Tissue	Pectoralis major muscle	60	1.06	1.15^{a}	0.71	1.53^{a}
	Gastrocnemius muscle	60	0.90	0.73^{b}	0.82	0.71^{b}
	SEM		0.11	0.08	0.06	0.19
	P-value		0.45	0.01	0.37	0.03
			P-value			
Interaction[2]	P×A		0.19	0.004	0.08	0.18
	P×T		0.87	0.005	0.22	0.16
	A×T		0.05	< 0.001	0.03	0.002
	P×A×T		0.04	< 0.001	0.04	0.20

[1]Means in a column within a variation without a common letter differ significantly ($P < 0.05$).
[2]P, A, and T represent effects of population, age, and tissue, respectively.

Interaction of population and tissue with population with age on MyoG mRNA

The differences between the two populations in *MyoG* for all time points were not significant ($P > 0.05$; Fig. 6). As shown in Figure 7, the expression of *MyoG* was the greatest in FGP, compared with other combinations ($P < 0.05$), and no significant differences were observed among the other combinations ($P > 0.05$).

Interaction of tissue and age on gene expression

There was an interaction of tissue and age on the expression of the four genes in PM and GM (Fig. 8). No significant differences were observed between the two tissues at any time point for the expression of *MyoG*, *IGF-1*, and *Pax7* ($P > 0.05$). *MyoG* expression in PM was greater on d 49 than days 1 and 70 ($P < 0.05$) (Fig. 8a). The expression of *Pax7* in PM on d 1 was greater than on d 70 ($P < 0.05$) (Fig. 8c).

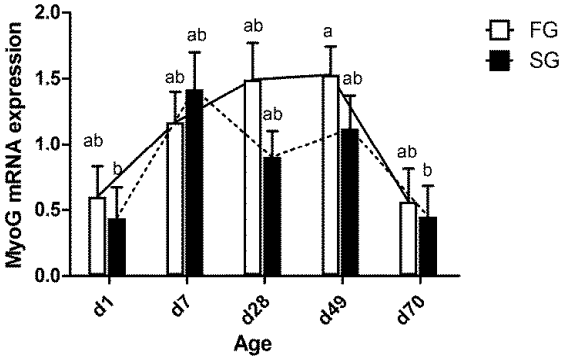

Fig. 6. Effect of population and age on expression of *MyoG* in the two populations on d 1, 7, 28, 49, and 70. Means without a common letter differ significantly ($P < 0.05$).

Interaction of population, age, and tissue on mRNA

No significant differences were observed between the tissues at the same time point for *MyoD* (Fig. 9a). On d 28, the expression of *MyoD* (Fig. 9a) and *MyoG* (Fig. 9b) in PM of FG peaked with respect to the other ages. Expression of *MyoG* in PM was greater than GM for FG on d 28 (P <0.05) (Fig. 9b). The expression of *MyoG* (Fig. 9b) and *MyoD* (Fig. 9a) in PM of FG initially increased, then decreased, and was greatest on d 28.

Other interaction effects on myofiber traits and gene expression were not significant.

DISCUSSION

The physiological characteristics of skeletal muscle, such as myofiber density and diameter, are important in chicken breeding and production (Chen *et al.*, 2013). The development of myofibers in poultry is artificially divided into two stages: incubation and post-hatch periods. During the incubation period, the myofiber precursor cells proliferate, then fuse into myotubes, and finally differentiate into myofibers (Picard *et al.*, 2002). In general, the total number of muscle fibers are constant during the post-hatch period (Baryshnikova *et al.*, 2007). Meanwhile, hypertrophy and the extension of myofibers decrease myofiber density during muscle development post-hatch (Wang *et al.*, 2017).

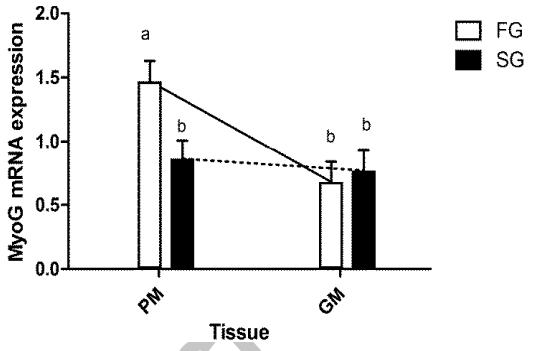

Fig. 7. Effects of population and tissue on expression of *MyoG* in PM and GM tissues. Means without a common letter differ significantly (P < 0.05).

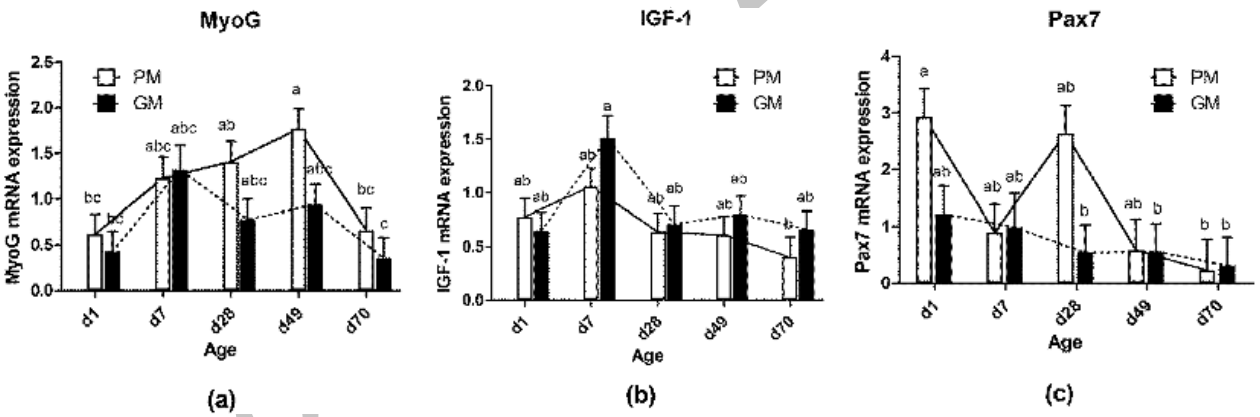

Fig. 8. Effect of age and tissue on gene expression in PM and GM on d 1, 7, 28, 49, and 70. (a) *MyoG*, (b) *IGF-1*, (c) *Pax7*. Means without a common letter differ significantly (P < 0.05).

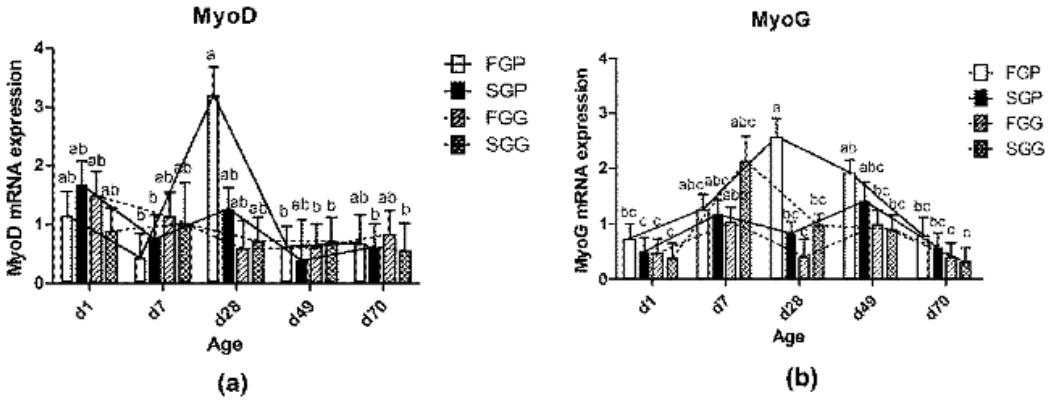

Fig. 9. Gene expression in the PM and GM of populations FG and SG on d 1, 7, 28, 49, and 70. (a) *MyoD*, (b) *MyoG*; FGP = PM

of FG, SGP = PM of SG, FGG = GM of FG, SGG = GM of SG. Means without a common letter differ significantly ($P < 0.05$).

Our results showed that muscle weight and myofiber diameter increased with time in PM and GM for FG and SG, while myofiber density was mostly unchanged, showing that myofiber density peaked initially and then was relatively stable until the end of the study, consistent with another chicken experiment (Chen *et al*., 2007).

Skeletal muscle fiber characteristics are affected by genetics. Several studies reported differences in carcass characteristics and meat quality between slow- and fast-growing breeds (Cassandro *et al.*, 2016; Verdiglione and Cassandro, 2013). Cobb broilers are fast-growing, have large breast meat yields, and reach the market weight on d 40 (Eldeeb *et al.*, 2006). However, HS1, like other breeds of Chinese meat-type chickens, are slow-growing and reaches market at 12 weeks or later. In the current study, the weights and fiber diameters of the skeletal muscle of Cobb increased at a greater rate than HS1 from d 28 to onward, which demonstrates that FG muscle develops faster. The myofiber diameter of fast- growing broiler is higher than slow-growing chickens due to the greater number of giant fibers. This fast growth rate may negatively impact on meat quality. Meat tenderness is negatively correlated with myofiber density; thus, the myofiber density of chickens should be considered in evaluating meat tenderness (An *et al.*, 2010). Our results revealed that the myofibers of FG were thicker than SG, thus the meat of FG may be considered to be tougher than SG.

The developmental profiles of different skeletal muscle tissues differed greatly (Ying *et al.*, 2016). A previous study reported that the leg muscle yield of quality chickens are greater than breast muscle yield (Sabbioni *et al.*, 2006). We found that the leg of SG was heavier than their breast on d 70. From d 1 to 7, the fiber density in PM was greater than that in GM in SG. When the density was stable, muscle growth depended on myofiber hypertrophy. The weights of the breast and leg of FG were similar. The fiber diameter in PM was larger than that in GM for FG on d 28. However, after d 49, the increasing tendencies in fiber diameter in PM and GM of FG was not different which indicates that the growth rate of PM was greater than GM in FG during the early post-hatch period. This is consistent with a previous study, which found that the muscle growth rate of breast was greater than leg in Cobbs (Abdulla *et al.*, 2017).

The GM fiber diameter of FG was thicker than PM and greater than GM in SG. Age is an essential factor affecting the development of skeletal muscle. When the effect of age was removed, myofiber diameter poorly described skeletal muscle development (Baéza *et al.*, 2012). The interaction effects of tissue and age indicated that the increase of myofiber diameter in chickens were different between the two muscle tissues for all ages.

Several studies (Pallafacchina *et al.*, 2013; Sacco *et al.*, 2008) reported that skeletal muscle satellite cells affected the development of muscle fiber dimension. The activation, proliferation, and differentiation of stem cells induce myofiber hypertrophy. When the myofiber matures, the satellite cells are in a relatively static state, which maintains the relative constancy of the histological characteristics of the myofiber, also, the development of satellite cells are affected by factors such as *MyoD* and myogenin (Pallafacchina *et al.*, 2010).

The expression of genes evaluated in this study always showed spatiotemporal change. *Pax7* plays a critical role during activation, proliferation, and differentiation. Increasing the expression of *Pax7* promotes satellite cell self-renewal (Craig *et al.*, 2008). In other words, a high expression of *Pax7* is always accompanied by growth of myofiber density. In the present study, the expression of *Pax7* in PM was time-dependent. *Pax7* expression in PM decreased with time and was accompanied by decreased PM fiber density. Moreover, no significant differences were observed between the PM and GM in terms of *Pax7* expression abundance and myofiber density.

Expression of *IGF-1*, *MyoG*, and *MyoD* were increased myofiber diameter and resulted in hypertrophy. *MyoD* function is also involved in myofiber-type transformation (Lee *et al.*, 2016; Sharma *et al.*, 2016; Wang *et al.*, 2013). In the current study, the expression of *MyoG* and *MyoD* in PM of FG were peaked on d 28, thereby suggesting that the age of 28 days is vital for skeletal muscle development. *MyoG* mRNA was peaked in PM on d 49. In different tissues of FG, the expression of *MyoG* was contrary to the increase of myofiber diameter. In conclusion, tissue was a significant factor influencing *MyoG* expression. *IGF-1* plays an essential role in heart and skeletal muscle development, which regulates the cell cycle, promoting cell fusion and protein synthesis (Clemmons, 2009), and increasing myofiber diameter and the rates of protein synthesis (Latres *et al.*, 2005). However, no prominent difference was observed between the fast- and slow- growing genetic chickens and between PM and GM muscle tissues. A previous study on broilers suggested that *IGF-1* mRNA is down-regulated with age in the breast, but the expression profile of *IGF-1* did not significantly change after d 7 (Saneyasu *et al.*, 2016). Moreover, the polymorphism of *IGF-1* is not associated with body weight in fast-growing chickens (Paswan *et al.*, 2013). Thus, we did not consider *IGF-1* to be a major gene affecting skeletal muscle in FG and SG.

CONCLUSION

Our results indicated that body weights and muscle expansion of slow- growing chickens were less than fast- growing chickens. The expression abundance of *MyoG* was affected by genetics, age, tissues, and their interactions. The expression profiles of *Pax7* were consistent with the growth trend of myofiber density, whereas *MyoG* and *MyoD* were associated with myofiber diameter changes. Overall, we revealed that body weight and muscle expansion of slow- growing chickens were less than fast- growing chickens. *MyoG* was a gene that contributes to phenotypic differences between the slow- and fast- growing chickens.

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Statement of conflict of interest

The authors declare no conflict of interest.

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