Pakistan Veterinary Journal

ISSN: 0253-8318 (PRINT), 2074-7764 (ONLINE)
Accessible at: www.pvj.com.pk

RESEARCH ARTICLE

Differential Expression Levels of Genes Related to Myogenesis During Embryogenesis of Quail and Chicken

Qian Ban, Yaowei Liang, Zongsheng Zhao[§]*, Xiaojun Liu[§] and Qingfeng Li

College of Animal Science and Technology, Shihezi University, Road Beisi, Shihezi 832003, Xinjiang, People's Republic of China
*Corresponding author: zhaozongsh@shzu.edu.cn

ARTICLE HISTORY

Received: June 15, 2012
Revised: January 23, 2013
Accepted: February 03, 2013

Key words:
Chicken
Embryo
Gene expression
Myogenesis
Quail

ABSTRACT

The present study was designed to investigate the expression dynamics of genes during myogenesis in quail and chicken. Real-time PCR was used to detect mRNA expressions of MyoD, MyoG, MLP and MSTN in breast muscle of quail and chicken embryos during the period of embryonic days E7-17. Results showed that expression profiles of each gene displayed similar trend in the experiment period between quail and chicken, however, the expression concentration between the two species differed at the same time detected. MyoD mRNA expression in quail was significantly lower in the early phase of the experiment period (E7-9) ($P<0.01$ on E7; $P<0.05$ on both E8 and E9). For MyoG and MLP, the mRNA expressions were both lower in quail than that in chicken during the experiment period. Additionally, the embryonic day when quail reached its peak expression was earlier than that in chicken (MyoG: quail E12 vs. chicken E13; MLP: quail E14 vs. chicken E15), and the peak expression for both in quail was significantly lower than that in chicken ($P<0.01$ for both). For MSTN, expression in quail was significantly higher in quail than that in chicken at each time detected ($P<0.01$). It is concluded that differential expression of these genes might or at least partially contributed to the different development of muscle development in quail and chicken.

To Cite This Article: Ban Q, Y Liang, Z Zhao, X Liu and Q Li, 2013. Differential expression levels of genes related to myogenesis during embryogenesis of quail and chicken. Pak Vet J, 33(3): 317-320.

INTRODUCTION

Muscle development, myogenesis, is a complex process, which can be divided into two phases: the embryonic and the postnatal phase. Compared with the poorly understood postnatal phase, the embryonic myogenesis received much more attention in the past decades either in domestic animals or in avian (Bentzinger *et al*., 2012). During the process of embryonic myogenesis, the myogenic progenitor cells (myoblasts) firstly proliferate and differentiate extensively, but then decrease because the number of myoblasts fused into multinucleated myotubes and myofibers, finally the muscle maintained its growth and maturation until postnatal (Davis and Fiorotto, 2009; Zhao *et al*., 2011).

It has been reported that a broad spectrum of genes regulated myogenesis during the embryonic phase, such as the myogenic regulatory factors (MRFs) including MyoD (Liu *et al*., 2011a), myogenin (MyoG) (Liu *et al*., 2011b), Myf5 (Braun *et al*., 1992) and MRF4 (Braun *et al*., 1990; Liu *et al*., 2011b), mystatin (MSTN) (McPherron and Lee, 1997; Huang *et al*., 2011; de Santis *et al*., 2012) and muscle LIM protein (MLP) (Kong *et al*., 1997; Swali *et al*., 2011). Extensive data on the expression pattern of these genes that regulate the development of muscle in embryogenesis have been obtained in avian like chicken (Gabriel *et al*., 2011; Saxena *et al*., 2007), duck (Li *et al*., 2010; Huang *et al*., 2011). However, there were few reports in the literature describing alterations in the expression pattern of these genes in quail myogenesis, although sequential activation of three myogenic regulatory genes during somite morphogenesis in quail embryos by in situ hybridization has been reported as early as 1992 (Pownall and Emerson, 1992). Furthermore, there was seldom comparison about the molecular genetic regulatory of myogenesis between chicken and quail, in other words, whether the expression profile of these genes in the two species has some differences is still not clear, although the quail (*Coturnix coturnix*) belongs to the same order (Galliformes) and

[§]These authors contributed equally to this work.

family (Phasianidae) as the chicken (*Gallus gallus*), and comparison studies for the two species, on expressions of gene related to other organs development during embryogenesis, like reproductive organ development during sex differentiation, have received much more attention, which is reviewed by Brunstrom *et al*. (2009). It is, therefore, necessary to understand the dynamics of genes during myogenesis between quail and chicken, which could be able to partially uncover the complex mechanism underlying muscle development in avian.

The present study, therefore, was designed to investigate the dynamics of gene expressions during myogenesis in quail and chicken. Real-time PCR was used to detect mRNA expressions of MyoD, MyoG, MLP and MSTN in breast muscle of quail and chicken embryos during the period of E7-17.

MATERIALS AND METHODS

Sampling: Fertilized eggs were obtained from 200 Yellow chicken (*Gallus gallus*) and 200 Korea quails (*Coturnix coturnix*), respectively, provided by the animal experiment station, Shihezi University. They were incubated in humidified atmosphere (60-70%) at 37.8±0.5°C (control incubation conditions). The embryo age was staged and measured in terms of the days of incubation. Whole embryos were collected through embryonic days (E) 7-17 (n=6 per day), during which, the breast muscle were isolated, frozen immediately in liquid nitrogen, and then stored at -80°C for RNA extraction.

RNA extraction and reverse transcription: Total RNA was isolated from the above tissues, according to standard Trizol extraction procedures. The quality of total RNA was examined by ethidium bromide stained denaturing agarose gel electrophoresis. The concentration of RNA was determined by a spectrophotometer. RNA samples were stored at -80°C prior to use. Total RNA of each sample (500ng) was reverse transcribed using the PrimeScript RT Master Mix (Perfect Real Time) (TaKaRa Biotechnology Co., Ltd. Dalian, China) according to the manufacturer's instructions.

Real-time PCR: The PCR primers were designed, according to the sequence of those genes in chicken in the GenBank (Table 1) by Primer premier 5.0 and synthesized by Beijing BGI Company, China. Real-time PCR condition was noted under Table 1. Each reaction was carried out in a total volume of 20 μL, consisting of 12.5 μL SYBR@ Premix Ex, 0.5 μL each primer, 2μL cDNA and 4.5 μL ddH_2O. Amplification reactions in triplicate for each sample were performed and the results were normalized to the β-actin gene expression level.

Statistical analysis: The relative mRNA expression level of genes was calculated by "normalized relative quantification" method, and statistical analysis was carried out using SPSS version 17.0. One-way ANOVA test and repeated measure of ANOVA were used for statistical analysis of normalized gene copy number, and differences were considered significant at $P<0.05$.

RESULTS

The mRNA expressions of MyoD, MyoG, MLP and MSTN gene in breast muscle of quail and chicken embryos at stages from E7-17 were detected by real time PCR method. Expression levels of these genes in quail and chicken were illustrated in Table 2 and Table 3, respectively. It can be clearly observed that there was a similar expression trend of MyoD mRNA between the two species: initial and also highest MyoD expression was found on E7, and then expression levels suffered a significant decline till the end of the experiment ($P<0.01$) (Table 2 and Table 3). During the period of E7-9, MyoD mRNA expressions in quail was significantly lower than that in chicken ($P<0.01$ On E7, $P<0.05$ on both E8 and E9). From E10 onward, the mRNA expressions of MyoD both in quail and chicken sustained their declining almost to the same level till E17, at which, their expressions dropped nearly to zero (Table 2 and Table 3).

Generally, the mRNA expression of MyoG showed the similar trend between the two species, although it was lower in quail (Table 2) than that in chicken (Table 3) at each time detected. During the whole period, MyoG levels rose from the initial stage (E7), after reaching the peak (quail at E12 and chicken at E13, respectively), levels declined significantly. Compared with chicken, the expression level at the peak was significantly lower than that in chicken ($P<0.01$).

Tables 2 and 3 also demonstrated the mRNA levels of MLP in breast muscle of quail and chicken embryos during periods of E7-17. It can be clearly observed that the expression level of MLP mRNA was different at each developmental stage detected, but there was a similar expression pattern between quail and chicken. From E7 to E12, MLP mRNA expressions, in both of them, remained lower levels, although there was a slight increase during this period, then, a suddenly dramatic rising expression was recorded on E13. The expression level of MLP in

Table 1: Primer sequences, GenBank accession number for the target genes, and conditions of Real-time PCR

RNA target	Primer sequence	Product size /bp	Annealing temperature (°C)	Acc. No.
MyoD	F: 5′GATTTCCACAGACAACTCCACAT3′ R: 5′GAATCTGGGCTCCACTGTCACT3′	116	55	FJ977569.1
MyoG	F: 5′GTGGGATGGTGATGCTGGAA3′ R: 5′TTGGAGAGGAGTGGGAAAGGA3′	109	55	FJ882411.1
MLP	F: 5′CTCACGAATCTGAAATCTACTGC3′ R: 5′GACATTTATCCACGTCAACCAT3′	213	56	XM_420911.2
MSTN	F: 5′ACAGTAGCGATGGCTCTTT3′ R: 5′CCGTTGTAGGTTTTTGGAC3′	207	55	AY448007.1
β-actin	F: 5′CTGTGCCCATCTATGAAGGCTA3′ R: 5′ATTTCTCTCTCGGCTGTGGTG3′	139	55	NM_205518.1

F: forward primer; R: reverse primer; Acc. No: GenBank accession number. Primers were designed according to the sequence of the target genes by there Acc. No. in GenBank.

Table 2: mRNA levels of MyoD, MyoG, MLP and MSTN in breast muscle of quail embryos during the period of embryonic days 7-17.

Days of embryogenesis	Genes			
	MyoD	MyoG	MLP	MSTN
E7	1.00±0.10^{a}	1.00±0.61^{a}	1.00±1.21^{a}	1.00±0.15^{ab}
E8	0.57±0.05^{b}	1.10±0.37^{a}	1.90±1.10^{a}	0.61±0.13^{ab}
E9	0.21±0.04^{c}	1.66±0.42^{ab}	3.47±0.84^{a}	1.12±0.02^{ab}
E10	0.19±0.07^{c}	2.11±0.39^{ab}	7.48±1.95^{a}	1.37±0.04^{b}
E11	0.16±012^{c}	2.78±0.39^{b}	5.66±3.46^{a}	1.52±0.04^{b}
E12	0.19±0.17^{c}	4.10±0.30^{c}	4.13±2.37^{a}	2.04±0.14^{c}
E13	0.11±0.09^{c}	3.39±0.91^{b}	47.95±9.60^{b}	2.46±0.22^{c}
E14	0.09±0.07^{c}	1.94±0.70^{ab}	81.20±1.40^{c}	2.69±0.11^{c}
E15	0.07±0.08^{c}	0.98±0.76^{a}	50.33±3.80^{b}	2.36±0.14^{c}
E16	0.05±0.05^{c}	0.54±0.55^{d}	57.81±6.55^{b}	2.48±0.13^{c}
E17	0.03±0.02^{c}	0.43±0.24^{d}	18.70±2.37^{d}	2.26±0.12^{c}

Each mRNA of these genes in breast muscle from quail embryos detected by Real-time PCR is displayed as a relative to β-actin. Each value is the mean±SE of six embryos. Values bearing different letters in a column differ significantly (P<0.05).

Table 3: mRNA levels of MyoD, MyoG, MLP and MSTN in breast muscle of chicken embryos during the period of embryonic days 7-17

Days of embryogenesis	Genes			
	MyoD	MyoG	MLP	MSTN
E7	1.80±0.14^{a}	1.44±0.39^{a}	5.94±1.21^{a}	0.03±0.04^{a}
E8	0.92±0.08^{b}	1.66±0.86^{a}	10.46±3.3^{a}	0.03±0.03^{a}
E9	0.43±0.14^{c}	3.47±0.30^{b}	10.75±0.8^{a}	0.07±0.06^{b}
E10	0.31±0.07^{c}	3.41±0.46^{b}	9.06±0.78^{a}	0.03±0.02^{a}
E11	0.22±0.08^{c}	4.61±0.70^{bc}	11.88±3.46^{a}	0.08±0.11^{b}
E12	0.23±0.05^{c}	6.36±0.38^{c}	13.45±2.37^{a}	0.16±0.09^{c}
E13	0.13±0.02^{d}	9.80±0.55^{d}	37.62±6.86^{b}	0.30±0.07^{d}
E14	0.05±0.03^{d}	3.00±0.24^{b}	70.00±3.51^{c}	0.30±0.13^{d}
E15	0.05±0.057^{d}	1.98±0.30^{a}	103.3±3.8^{d}	0.31±0.12^{d}
E16	0.04±0.04^{d}	0.72±0.55^{e}	75.24±10.91^{c}	0.34±0.11^{d}
E17	0.04±0.03^{d}	0.45±0.42^{e}	64.30±4.74^{c}	0.36±0.11^{d}

Each mRNA of these genes in breast muscle from chicken embryos detected by Real-time PCR is displayed as a relative to β-actin. Each value is the mean±SE of six embryos. Values bearing different letters in a column differ significantly (P<0.05).

quail reached the top at the stage E14, which was just before the day (E15) when chicken reached its peak expression. Moreover, there was a significant difference in terms of the peak expression between the two species (P<0.05). After reaching its respective peak, MLP levels declined in both of them.

We can also see that at each stage, MSTN expression level in quail (Table 2) was significantly higher than that in chicken (Table 3) (P<0.01). In quail, initial MSTN expression was found on E7, after declining on E8, level then increased gradually and reached the top at the stage E14, and thereafter plateaued (Table 2). In chicken, MSTN expression increased manifolds from E11 onwards as compared that from E7 to E10. Peak expression was noticed at E13, which was almost maintained at this level until E17 (Table 3). The late phase of embryogenesis (E13-17) witnessed a relatively stable MSTN expression level in both quail and chicken, although it was significantly higher in quail as compared with chicken during this period.

DISCUSSION

MyoD is an important member in the myogenic regulatory factors (MRFs) gene family, which includes MyoD1, Myf5, Mrf and Myogenin. Of these factors, MyoD is the first to be expressed, which act genetically upstream and is of significant importance to the determination and proliferation of myogenic progenitor cells, especially to specify myoblasts for terminal differentiation (Bentzinger *et al*., 2012). In the present study, we examined the mRNA expressions of MyoD gene in breast muscle of quail and chicken embryos in the stages of embryogenesis (E7-17). MyoD expression showed a downward trend in both of them, with the expression peak occurring at the initial stage (E7). It is known that a secondary wave of myogenesis occurs in birds after sixth day of incubation (E6) (Fredette and Landmesser, 1991). In this study, the period of E7-17 was chose, and highest MyoD expression was recorded on E7 for both of them, suggesting that the high MyoD expressions probably play an important role in the early phase of myogenesis. In addition, we found that the initial expression of MyoD was significantly lower in quail than that in chicken, although the similar expression trend was observed thereafter. This difference might be due to the reason that on E7, quail and chicken might be at different phase of muscle development, respectively, thereby requiring different MyoD concentrations to involve in the process of myoblast proliferation and differentiation.

Compared with MyoD, MyoG is more directly involved in the myoblasts differentiation process and trigger the expression of myotube specific genes in the myogenesis (Bentzinger *et al*., 2012). In this study, we detected the mRNA levels of MyoG in breast muscle of quail and chicken embryos during periods of E7-17. MyoG mRNA expression displayed a similar expression pattern between the two species, although it was different at each development stage. Expression of MyoG mRNA rose from the initial stage (E7), after reaching its respective peak (quail at E12 and chicken at E13), it declined significantly. The time of peak expression for MyoG in quail and chicken is consistent with the findings reported by O'Neill (1987), at which, a large number of myotubes could be observed in the breast muscle. It is inferred that MyoG might directly act on the formation of myotubes, or indirectly act on by triggering the expression of myotubes specific genes.

MLP belongs to the LIM superfamily of proteins, which plays important roles in a variety of cellular functions. Arber *et al*. (1994) found that in chicken and rodent muscle cells, MLP is not detected in proliferating myoblasts but is up-regulated during terminal differentiation. In this study, we detected the mRNA expression levels of MLP in breast muscle of quail and chicken embryos during periods of E7-17.We found that in both quail and chicken, MLP mRNA expressions remained lower levels from E7 to E12, but from E12 onward, they rose dramatically, reaching their respective peak, and then still remain their high levels, although a slight decline occurred after the peak. Our findings thus indicated that MLP might be involved in the later stages of muscle development, especially in the formation of myotubes, because from E12 onward, a large number of myotubes began to fuse. This speculation is consistent with the findings reported by previous workers (Arber *et al*., 1994). In addition, quail and chicken reached to its respective peak expression at different stage. Moreover, significant difference occurred in terms of their peak expression between them. It is inferred that the different MLP expressions at different time might influence the rate of mytube fusion and the thickness of muscle fiber,

thereby resulting in the differences of muscle development between them.

It has been demonstrated in the previous study that MSTN is not the only a major determinant of muscle mass, but also influences early embryogenesis in avian (Saxena *et al*., 2007). In the present study, the expression of MSTN gene in breast muscle during embryogenesis (E7-17) of quail and chicken was detected, respectively. We found that in the early stage of embryogenesis, MSTN expression displayed relatively lower and plateaued levels, especially for chicken, while in the late phase of (E13-17) embryogenesis, levels rose highly and almost remained stable for both of them. Saxena *et al*. (2007) reported that higher and almost static MSTN expression was noticed in biceps femoris muscle of Indian broilers during the entire period of myogenesis (E7–E18). Compared with our study, the difference in expression level may be due to the different development between leg muscle and breast muscle in chicken. In addition, in the present study, we found that MSTN mRNA expression reached its first peak on E9 in chicken, which coincides with the periods of primary and secondary muscle fiber formation in chick embryos. While in quail, the first peak expression of MSTN was observed on E7. Compared with chicken, this difference could be due to the different development for embryonic myogenesis between quail and chicken, as the day for hatch out in quail is earlier than that in chicken for about 2-3days, resulting in the earlier muscle development occurred in quail. Hence, MSTN expression reaches its first peak is earlier than that in chicken. Furthermore, MSTN expression differed significantly between quail and chicken, which may be due to the different species in avian.

Conclusion: Expression profiles of each gene detected in the present study displayed similar trend in the experiment period between quail and chicken, however, the expression concentration between them differed at the same time point detected. It is inferred differential expression of these genes might or at least partially caused the different development of muscle development in quail and chicken.

REFERENCES

Arber S, G Halder and P Caroni, 1994. Muscle LIM protein, a novel essential regulator of myogenesis, promotes myogenic differentiation. Cell, 79: 221-231.

Bentzinger CF, YX Wang and MA Rudnicki, 2012. Building muscle: Molecular regulation of myogenesis. Cold Spring Harb Perspect Biol, 4: a008342.

Braun T, MA Rudnicki, HH Arnold and R Jaenisch, 1992. Targeted inactivation of the muscle regulatory gene Myf-5 results in abnormal rib development and perinatal death. Cell, 71: 369-382.

Braun T, E Bober, B Winter, N Rosenthal and HH Arnold, 1990. Myf-6, a new member of the human gene family of myogenic determination factors: Evidence for a gene cluster on chromosome 12. EMBO J, 9: 821-831.

Brunstrom B, J Axelsson, A Mattsson, and K Halldin, 2009. Effects of estrogens on sex differentiation in Japanese quail and chicken. Gen Comp Endocrinol, 163: 97-103.

Davis TA and ML Fiorotto, 2009. Regulation of muscle growth in neonates. Curr Opin Clin Nutr Metab Care, 12: 78-85.

de Santis C, GB Gomes and DR Jerry, 2012. Abundance of myostatin gene transcripts and their correlation with muscle hypertrophy during the development of barramundi, *Lates calcarifer*. Comp Biochem Phys B Biochem Mol Biol, 163: 101-107.

Fredette BJ and LT Landmesser, 1991. A reevaluation of the role of innervation in primary and secondary myogenesis in developing chick muscle. Dev Biol, 143: 19-35.

Gabriel JE, HJ Alves, MF Do Rosário, A Secatto, LL Coutinho and M Macari, 2011. Abundance of MyoD and myostatin transcripts in chicken embryos submitted to distinct incubation temperatures and timing exposures. Braz J Biol, 71: 563-564.

Huang KL, JW Wang, CC Han, HH Liu, L Li, F Dai, Z Pan, F Xu, H He and H Xu, 2011. Developmental expression and alternative splicing of the duck myostatin gene. Comp Biochem Physiol Part D Genomics Proteomics, 6: 238-243.

Kong Y, MJ Flick, AJ Kudla and SF Konieczny, 1997. Muscle LIM protein promotes myogenesis by enhancing the activity of MyoD. Mol Cell Biol, 17: 4750-4760.

Li L, HH Liu, F Xu, JM Si, J Jia and JW Wang, 2010. MyoD expression profile and developmental differences of leg and breast muscle in Peking duck (Anas platyrhynchos Domestica) during embryonic to neonatal stages. Micron, 41: 847-852.

Liu HH, JW Wang, L Li, CC Han, KL Huang, JM Si, H He and F Xu, 2011a. Molecular evolutionary analysis of the duck MYOD gene family and its differential expression pattern in breast muscle development. Br Poult Sci, 52: 423-431.

Liu HH, JW Wang, X Chen, RP Zhang, HY Yu, HB Jin, L Li and CC Han, 2011b. In ovo administration of rhIGF-1 to duck eggs affects the expression of myogenic transcription factors and muscle mass during late embryo development. J Appl Physiol, 111: 1789-1797.

McPherron AC and SJ Lee, 1997. Double muscling in cattle due to mutations the myostatin gene. Proc Natl Acad Sci USA, 94: 12457-12461.

O'Neill MC, 1987. Growth and differentiation during myogenesis in the chick embryo. Dev Biol, 120: 465-480.

Pownall ME and CP Emerson Jr, 1992. Sequential activation of three myogenic regulatory genes during somite morphogenesis in quail embryos. Dev Biol, 151: 67-79.

Saxena VK, NR Sundaresan, F Malik, KA Ahmed, M Saxena, S Kumar, PV Nandedkar and RV Singh, 2007. Temporal expression of transforming growth factor-beta2 and myostatin mRNA during embryonic myogenesis in Indian broilers. Res Vet Sci, 82: 50-53.

Swali A, S McMullen, H Hayes, L Gambling, HJ McArdle and SC Langley-Evans, 2011. Cell Cycle regulation and cytoskeletal remodelling are critical processes in the nutritional programming of embryonic development. PLoS ONE, 6: e23189.

Zhao X, D Mo, A Li, W Gong, S Xiao, Y Zhang, L Qin, Y Niu, Y Guo, X Liu, P Cong, Z He, C Wang, J Li and Y Chen, 2011. Comparative analysis by sequencing of transcriptomes during skeletal muscle development between pig breeds differing in muscle growth rate and fatness. PLoS ONE, 6: e19774.