MYOSIN HEAVY CHAIN TYPES AND EXPRESSION LEVELS OF MYOSTATIN AND MYOGENIN GENES IN MUSCLE OF TWO RABBIT BREEDS

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ABSTRACT

The aim of the present study was to compare fiber type compositions in terms of Myosin Heavy Chain (*MyHC*) and expression levels of myostatin (*MSTN*) and myogenin (*MyoG*) in longissimus dorsi of two rabbit breeds with different body sizes. Longissimus dorsi samples of Qixin rabbits (n=12) and German New Zealand of ZIKA rabbits (n=12) at 84d after birth (marketing age) were collected. Quantitative real-time PCR analysis revealed four types of fiber in longissimus dorsi, and *MyHC-1* and *MyHC-2D* were the major types. No significant difference was observed in the proportions of *MyHC* isoforms in longissimus dorsi between the two breeds. In addition, the two rabbit breeds at 84d had showed non-significant mRNA differences in levels of *MSTN* and *MyoG* in longissimus dorsi. These results suggest that *MyHC* types, *MSTN* and *MyoG* expression may not directly associated with the different body sizes of the two rabbit breeds studied.

Key words: Rabbit, myosin heavy chain, myostatin, myogenin, gene expression.

INTRODUCTION

Muscle fiber type is a research focus in livestock science (Korfage *et al*., 2009; Francisco *et al*., 2011). The myosin heavy chain (*MyHC*) is one of the major structural and contracting proteins of muscle. Consequently, muscle fibers can be classified by their *MyHC* isoform contents, and may be affected by factors such as gender, age, postnatal development, muscle function, and genetic heritage (McKoy *et al*., 1998; Korfage *et al*., 2009). The two rabbit breeds in this study showed significant body weight difference (>6%) at marketing age. We hypothesized that rabbit breeds with different growth rates may vary in their muscle fiber type as well as myostatin (*MSTN*) and myogenin (*MyoG*) gene levels in longissimus dorsi, Since *MSTN* is a secreted growth factor predominately expressed in skeletal muscle that negatively regulates skeletal muscle mass (Lee, 2004), while *MyoG* is involved in many important processes such as myofiber cell differentiation (Knapp *et al*., 2006), therefore, these genes might affect the growth of rabbit muscles. At present, reports on the relationship between muscle growth and the expression levels of these genes are not available in rabbit.

MATERIALS AND METHODS

Animals and experimental design

The experimental rabbits (*Oryctolagus cuniculus*) included 12 Qixin (A) and 12 German New Zealand of ZIKA rabbits (N), these rabbits (half are male) were fed on the same commercial diet. All rabbits were selected randomly and killed 12 h after the last meal at 84 days of age. Longissimus dorsi adjacent to the last rib level was collected from the left-half carcasses and stored in liquid nitrogen. Body weights of the experimental rabbits were recorded at 40d and 84d after birth. The experiment was conducted according to the National Institutes of Health NIH Guidelines and National Research Council's publication “Guide for Care and Use of Laboratory Animals”.

Chemical Analyses

RNA preparation

Total RNA was extracted from longissimus dorsi of rabbits using Trizol reagent (Invitrogen, USA) according to the manual instruction, and treated with DNase to remove genomic DNA contamination. The quality of RNA was examined by agarose electrophoresis and spectrometry. The first stranded cDNA was synthesized by RevertAid^{TM} First Strand cDNA Synthesis Kit (Ferments).

Quantitative real-time RT-PCR analysis of target genes

Quantitative real-time PCR was developed to assay mRNA levels of the target genes and housekeeping gene of Glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*) listed in Table 1. The PCR primers were designed according to mRNA sequences in GenBank (Table 1) or primers from literatures (Barjot *et al*., 1995). RT-PCR mixture (20 μL) contained 1 μL of RT reaction mix, 10μL of SYBR® Premix Ex Taq ^{TM} (2×) (TaKaRa, China), 0.6μL of 10 μmol/L each of primers and ultra-pure water to 20μL. Reactions were run on a fluorescence iCycler (Bio-Rad). The PCR conditions were as follows: 94°C for 90 s; 43 cycles of 95°C for 15s, annealing temperature of the primers (Table 1) for 20s, 72°C for 15s. Each sample was assayed in duplicate. The PCR efficiencies were from 95% to 105%, with excellent linearity. The standard curves were obtained using purified plasmid. The threshold cycle (Ct) from PCR was analyzed using the $2^{-\Delta\Delta Ct}$ method (Livak and Schmittgen, 2001). Changes in expressions of the target genes were normalized by the mRNA measurement of *GAPDH*.

Statistical analysis

Data of body weights and gene expression levels were analyzed using Statistical Package for the Social Science (SPSS 17.0). Values were expressed as Mean±SE. The significance of the mRNA levels of target genes and body weights were evaluated using independent-sample *t*-test and significance level set at $P<0.05$.

Table 1: Primer information for RT-PCR

Genes	Reference Sequence	Primers	Annealing temp.(°C)	Amlpicon size (bp)
MyHC-1	XM_002718960	F: GCTGGTACTGTGGACTACAACA R: GCTGACACGGTCTGAAAGGA	62	200
MyHC-2A	XM_002718950	F: CAGGCTTCAGGATTTGGT R: GACCTGGGACTCAGCAAT	56	166
MyHC-2B	XM_002718957	F: AGAGGCTGAGGAACAATCCA R: ACTTGATGCACAAGGTAGTG	58	236
MyHC-2D	OCU32574	F: GGCTGTCAAAGGTCTACGC R: CATTGTTCCTCCGCTTCC	59	162
MSTN	GU244569.1	F: AGAGCATTGATGTGAAGA R: GGAAGGTTACAGCAAGAT	51	114
MYOG	FJ605115.1	F: TCTACGACGGGGAGAACTACCT R: CTGACTTCCTCTTACACACCTTGC	60	186
GAPDH	NM_001082253.1	F: AGAGCACCAGAGGAGGACG R:TGGGATGGAAACTGTGAAGAG	59	105

RESULTS AND DISCUSSION

The body weights of German New Zealand of ZIKA rabbits at 40d and 84d after birth were higher ($P<0.01$) that those of Qixin rabbits (Table 2). No difference was observed between male and female rabbits of the same breed. Additionally, the daily gain from 40d and 84d after birth was not significant, indicating that their body weight difference was mainly determined within 40d after birth.

The four types of *MyHC* fiber screened were detected in rabbit longissimus dorsi by real-time PCR, *MyHC-1* and *MyHC-2D* were the major types in both breeds (Figure 1). No significant difference was observed in the proportions of *MyHC* isoforms in longissimus dorsi between Qixin and German New Zealand of ZIKA rabbits. McKoy et al. (1998) observed that *MyHC* gene expression at the mRNA level correlated strongly to those of protein level. Thus our present result on *MyHC* is regarded as the actual levels of the *MyHC* types. In addition, we detected no difference in mRNA levels of *MSTN* and *MyoG* in longissimus dorsi of the two rabbit breeds (data not shown).

Table 2: Body weight (g) of the two rabbit breeds

Rabbit breed	Body weight at 40 d	Body weight at 84 d
German New Zealand of ZIKA rabbit (N)	968.5±10.1	2235.0±9.5
Qixin rabbit (A)	847.7±7.5*	2082.7±8.5*

*$P<0.01$, compared between the two breeds at the same age. The P values are 0.002 and 0.00006 for body weight at 40d and 84d, respectively.

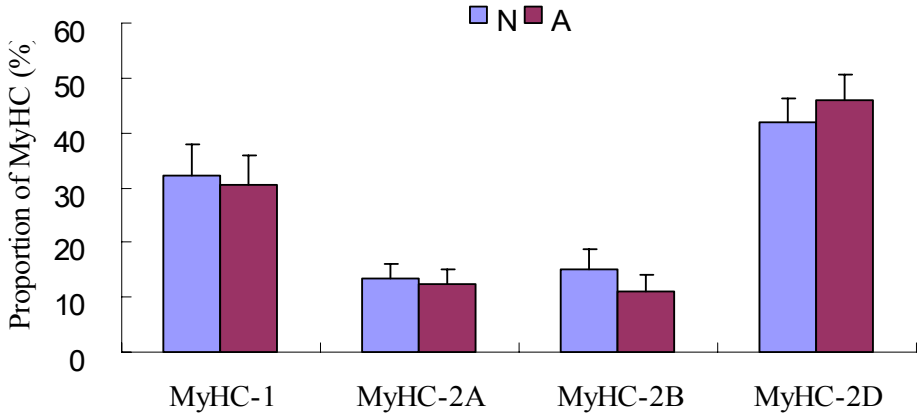

Figure 1: Proportion of *MyHC* isoforms in rabbit longissimus dorsi at 84 days of age

The similar *MyHC* isoform proportions in longissimus dorsi of the two rabbit breeds indicate that body size and genetic background have no significant effects on muscle fiber types at 84d after birth. In recent years, many candidate genes have been proposed for muscle growth and meat quality (De Koning *et al*., 1999; Hoashi *et al*., 2008). *MSTN* and *MyoG* are regarded as candidate genes related to muscle growth and development (Koohmaraie 1996; Knapp *et al*., 2006). The current study reveals that both genes are not the main determinant for rabbit growth around 84d after birth. In conclusion, our results suggest that *MyHC* types, *MSTN* and *MyoG* may not directly correlate with the different body sizes of the two rabbit breeds studied.

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