
EXPRESSION PATTERNS OF TWO GENES ASSOCIATED WITH ADIPOSE DEPOSITION DURING GROWTH AND DEVELOPMENT IN TWO RABBIT BREEDS.

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EXPRESSION PATTERNS OF TWO GENES ASSOCIATED WITH ADIPOSE DEPOSITION DURING GROWTH AND DEVELOPMENT IN TWO RABBIT BREEDS

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ABSTRACT
Fatty acid synthase (FAS) and Carnitine palmitoyltransferase1 (CPT1) are the most important enzymes that affect adipose deposition. In this study, we estimated the relative expression of FAS and CPT1 gene at different ages (14, 35, 56, 70 and 84 days) in the Germany great line of ZIKA rabbit and in the Californian rabbit. The expression pattern of FAS gene was similar in the two breeds, but lower for the Germany great line of ZIKA rabbits than Californian rabbits from 14d to 84d. The expression patterns of CPT1 gene was basically the same in the two rabbit breeds from 56d to 84d, while the opposite pattern from 14d to 56d was observed. These results indicate that the expression levels of FAS and CPT1 gene varies at different development stages in the two rabbit lines and provides the basic data for further study on the regulation mechanism of adipose deposition in rabbits.

Key words: Rabbit, FAS, CPT1, Adipose deposition, Gene expression.

INTRODUCTION
The intramuscular fat content (IMF) is closely related with the tenderness, juiciness and flavor of the muscle, and it is a main factor affecting meat quality traits (Yong et al., 2005). Research is focused on maintaining the appropriate level of IMF while keeping a rapid growth rate. The adipose deposition is a homeostasis process on fat synthesis and catabolism. Therefore, it is very important to study the fat synthesis and catabolism for understanding dynamics of adipose deposition. Fatty acid synthase (FAS) and Carnitine palmitoyltransferase1 (CPT1) are the most important enzymes that affect adipose deposition because they participate on fat synthesis and catabolism. FAS plays an important role in the de novo lipogenesis in mammals. It is one of the key enzymes in the conversion of acetyl-CoA and malonyl-CoA to triglycerol (Clay et al., 1997; Stuart et al., 2003). CPT1 is a key factor in the oxidation of long-chain acids because it catalyzes their transport through the mitochondria membrane (Kerner et al., 2000; Virmani et al., 2015). This study aimed to investigate the molecular basis of adipose deposition in rabbit. A Q-PCR method has been used to detect mRNA expression level of FAS and CPT1 in muscle during growth and development in two rabbit breeds.

MATERIALS AND METHODS
Animals And Experimental Design
The experimental rabbits included 100 Germany great line of ZIKA rabbits (G, 50 males and 50 females) and 100 Californian rabbits (U, 50 males and 50 females). Rabbits were fed on the same commercial diet. Six rabbits of each group were selected randomly and slaughtered at 14, 35, 56, 70 and 84 days of age. Longissimus muscles were collected from the left-half carcasses and stored in liquid nitrogen. All experiments were conducted according to the NIH and National Research Council's publication “Guide for Care and Use of Laboratory Animals”.
Chemical Analyses

RNA preparation
Total RNA was extracted from *longissimus* muscles using Trizol reagent (Invitrogen, USA) according to the manual instruction. The purity and concentration of total RNA were measured with a spectrophotometer at 260 and 280 nm. Absorption ratios (260/280 nm) of all samples ranged from 1.8 to 2.0. The first stranded cDNA was synthesized by RevertAidTM First Strand cDNA Synthesis Kit (Ferments) following the manual instruction, and stored at -20°C.

Quantitative real-time RT-PCR analysis of target genes
Quantitative real-time RT-PCR was developed to assay mRNA levels of the target genes and the reference genes (GAPDH) listed in Table 1. The PCR primers were designed according to mRNA sequences in GenBank (Table 1). RT-PCR mixture (20 µL) contained 1 µL of RT reaction mix, 10µL of SYBR® Premix Ex Taq TM (2x) (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 threshold cycle (Ct) from RT-PCR was analyzed using the $2^{-\Delta\Delta Ct}$ method (Livak and Schmittgen, 2001). Changes in the expression of target genes were normalized by the geometric mean of the mRNA measurements of GAPDH in the same sample.

<table>
<thead>
<tr>
<th>Table 1: Primer information for RT-PCR</th>
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<tr>
<td>Genes</td>
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<tr>
<td>FAS</td>
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<td>CPT1</td>
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<td>GAPDH</td>
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Statistical Analysis
Data 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 were evaluated using independent-sample t-test and significance level was set at P<0.05.

RESULTS AND DISCUSSION
The expression levels of FAS and CPT1 gene were surveyed by real-time PCR. The results showed that the FAS expression level was the lowest at 14d and increased during growth and development from 14d to 84d in the two rabbit breeds (Fig 1). The analysis showed that the expression of FAS mRNA at 70d in G rabbits was not significantly different from the U rabbits. However, FAS mRNA expression was significantly lower for G than for U rabbits at the other ages.
Figure 1. Relative mRNA expression of the FAS gene in longissimus muscles of Germany great line of ZIKA (G) and Californian (U) rabbits at different ages. The expression level of the FAS gene in G at 14 days was arbitrarily set to 1.0. The same superscript letter indicates no significant differences among different ages within each rabbit breed while different letters indicate significant difference at 5% (small letter) or 1% (capital letter) level; single star”*” and double stars”**” indicates significant difference at 5% or 1% level, respectively, between breeds at the same age.

Figure 2. Relative mRNA expression of the CPT1 gene in longissimus muscles of Germany great line of ZIKA (G) and Californian (U) rabbits at different ages. The expression level of the CPT1 gene in G at 14 days was arbitrarily set to 1.0. The same superscript letter indicates no significant differences among different ages within each rabbit breed while different capital letters indicate significant difference at 1% level; single star”*” and double stars”**” indicates significant difference at 5% or 1% level, respectively, between breeds at the same age.

For expression levels of CPT1 mRNA (Fig 2), there were some differences between the two breeds. G rabbits exhibited ‘rise-decline’ developmental changes from 14d-84d, being at 84d significantly lower than on the other days. In U rabbits, CPT1 mRNA levels exhibited ‘decline-rise-decline’ developmental changes, being at 84d the lowest level than at 14d (P<0.01). When the two breeds were compared, CPT1 mRNA levels in G rabbits on 35d, 70d and 84d were significantly higher than that of U rabbits.

It is well known that the intramuscular fat (IMF) accumulation is affected by many factors, such as breed, age, nutrition and environmental factors (Katsumata et al., 2011; Suzuki et al., 2009). It has been showed in other studies that supplementary dietary protein increases intramuscular fat and the FAS enzyme activity, while CPT1 activity decreases (Zhang et al., 2014). Another study shows that Japanese black and
Holstein steers kept under equivalent conditions of high energy intake result in large differences in IMF accumulation in *longissimus* muscle. In recent years, many candidate genes have been proposed for adipogenesis and fat metabolism. FAS and CPT1 are regarded as candidate genes related to IMF accumulation, playing important roles in accumulation of IMF (Shirouchi et al., 2014; Zhang et al., 2014). Our experiment indicates that these two genes are differentially expressed in the *longissimus* muscles of two rabbit breeds.

**CONCLUSIONS**

In summary, we can conclude that the expression patterns of FAS gene are similar in the two rabbit breeds. The expression patterns of CPT1 gene are basically similar from 56d to 84d, but the opposite pattern is observed from 14d to 56d. To some extent, these results indicate that the expression levels of FAS and CPT1 genes show differential expression at different development stages in the two rabbit breeds. This study provides the basic data for further research on the regulated mechanism of adipose deposition in the rabbit.

**ACKNOWLEDGEMENTS**

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