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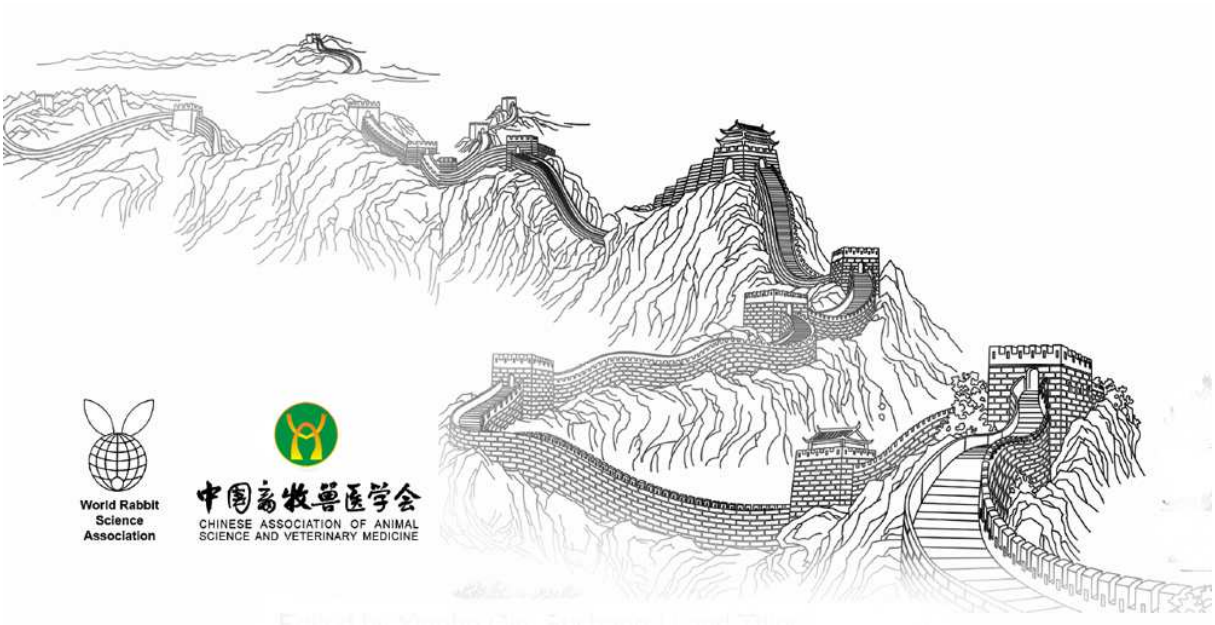

EXPRESSION PATTERNS OF TWO GENES ASSOCIATED WITH ADIPOSE DEPOSITION DURING GROWTH AND DEVELOPMENT IN TWO RABBIT BREEDS

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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 expressional 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 (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 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 1: Primer information for RT-PCR

Genes	Reference Sequence	Primers	Annealing temp(°C)	Amlpicon Size (bp)
FAS	KF201292	F: CCAACTACGGCTTTGCCAACTCC R: CAGGTCACGAATGCCCAGGATGT	62	329
CPT1	XM_002724092	F: TGAGCCCTGGAGGTTGT R: GAACTTGGAGGAAATGTGG	56	184
GAPDH	NM_001082253.1	F: AGAGCACCAGAGGAGGACG R:TGGGATGGAAACTGTGAAGAG	59	105

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 of U rabbits at the other ages.

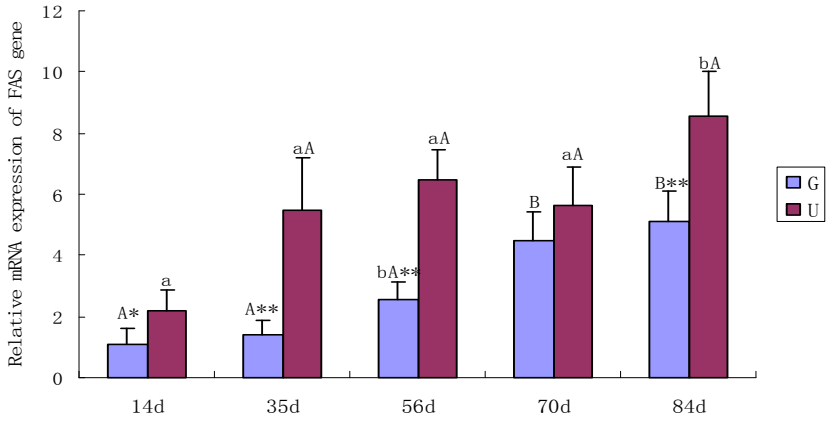

Figure .1 Relative mRNA expression of the FAS gene in *longissimus* muscles of Germany great line of ZIKA (G) and Californian (U) rabbits at different ages. The expression level of the FAS gene in G at 14 days was arbitrarily set to 1.0. The same superscript letter indicates no significant differences 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.

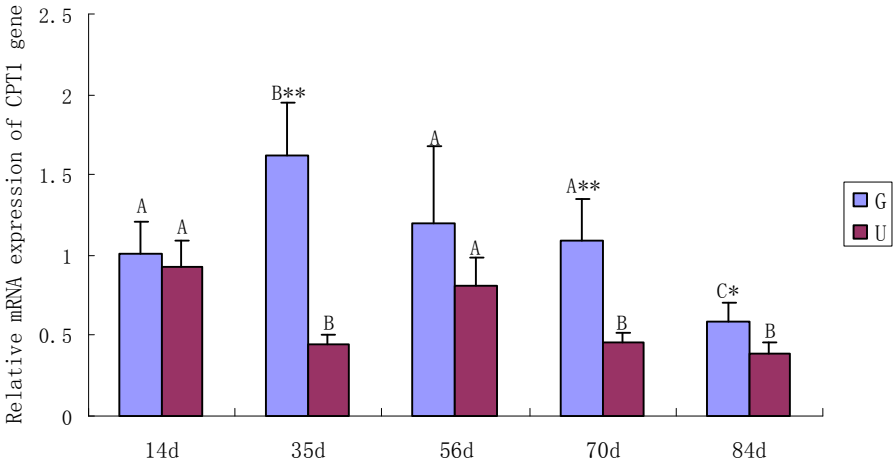

Figure.2 Relative mRNA expression of the CPT1 gene in *longissimus* muscles of Germany great line of ZIKA (G) and Californian (U) rabbits at different ages. The expression level of the CPT1 gene in G at 14 days was arbitrarily set to 1.0. The same superscript letter indicates no significant differences 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 activitiy 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 expressional 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.

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REFERENCES

Katsumata M. 2011. Promotion of intramuscular fat accumulation in porcine muscle by nutritional regulation. *Anim Sci J., 82(1),* 17-25.

Kerner J., Hoppel C. 2000. Fatty acid import into mitochondria. *Biochim Biophys Acta, 26(1), 1-17.*

Kristophe J Karami., John Coppola., Karlhik Krishnamrthy., et al. 2006. Effect of food deprivation and hormones of glucose homeostasis on the acetyl CoA carboxylase activity in mouse brain: a potential role of acc in the regulation of energy balance. *Nutrition & metabolism, 3, 1-9.*

Livak K.J., Schmittgen T.D. 2001. Analysis of relative gene expression data using real-time quantitative PCR and the 2-ΔΔCt method. *Methods, 25, 402-408.*

Shirouchi B., Albrecht E., Nuernberg G., Maak S., Olavanh S., Nakamura Y., Sato M., Gotoh T., Nuernberg K. 2014. Fatty acid profiles and adipogenic gene expression of various fat depots in Japanese Black and Holstein steers. *Meat Sci, 96(1), 157-164.*

Suzuki K., Inomata K., Katoh K., Kadowaki H., Shibata T. 2009. Genetic correlations among carcass cross-sectional fat area ratios, production traits, intramuscular fat, and serum leptin concentration in Duroc pigs. *J Anim Sci., 87(7), 2209-2215.*

Virmani A., Pinto L., Bauermann O., Zerelli S., Diedenhofen A., Binienda Z K., Ali S F., Van der Leij F R. 2015. The Carnitine Palmitoyl Transferase(CPT) System and Possible Relevance for Neuropsychiatric and Neurological Conditions. *Mol Neurobiol, 52(2), 826-836.*

Yong H W., Keren A B., Antonio R., Gregory S., Masaaki T., Sean M., William M., Hideyuki M., Kenji O., Sigrid A L. 2005. Transcriptional profiling of skeletal muscle tissue from two breeds of cattle. *Mammalian Genome, 16, 20l-210.*

Zhang H B., Wang Z S., Peng Q H., Tan C., Zou H W. 2014. Effects of different levels of protein supplementary diet on gene expressions related to intramuscular deposition in early-weaned yaks. *Anim Sci J., 85(4), 411-419.*

Zhang Y F., Yuan Z Q., Song D G., Zhou X H., Wang Y Z. 2014. Effects of cannabinoid receptor 1 (brain) on lipid accumulation by transcriptional control of CPT1A and CPT1B. *Anim Genet, 45(1), 38-47.*

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