

DEVELOPMENT REGULARITY OF WOOL FIBER AND EXPRESSION CHARACTERISTICS OF KERATIN ASSOCIATED PROTEINS GENE IN THREE RABBIT BREEDS

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Abstract

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This study measured the length of wool fiber of three rabbit breeds (including Fujian Yellow rabbit, German Angora rabbit and American Rex rabbit) in different growth period Respectively. We got 4 data sets of different positions, which were neck, back, buttock and abdominal. Real-time fluorescence quantitative PCR method was used to analyse the expression characteristics of KAP 3.1 and KAP 3.3 gene in back and buttock at the same time. The result of wool staple length indicated that the growth speed of wool fiber in Fujian Yellow rabbit and American Rex rabbit was higher than German Angora rabbit before 2 weeks old, but the wool length of American Rex rabbit was longer than the other two breeds after 4 weeks old. The result of RT-PCR showed that the expression of KAP3.1 gene had a high level in all breeds when it was newborn, and expression in Fujian Yellow rabbit and American Rex rabbit was higher than Angora rabbit. The expressions increased slowly in general, and the characteristics were similar between different sexes and positions. The expression of KAP3.1 gene in Fujian Yellow rabbit and American Rex rabbit reduced when it was 4 weeks and 12 weeks old, moreover, the reduction of back in American Rex rabbit was higher than Fujian Yellow rabbit. The expression characteristic of KAP3.3 gene was basically identical to KAP3.1 gene. We might predict that the development of wool fiber length was associated with KAP3.1 and KAP3.3 gene.

Key words: Rabbit; Skin; Wool fiber; KAP gene; RT-PCR; Expression characteristics

Introduction

As an important component of wool fiber, Keratin Proteins accounted for 65%-95% of the total weight of wool fibers (Zhang H.L. et al., 2001). There were two types, Intermediate Filament (IF) and Keratin Association Protein(KAP) (Purvis and Franklin, 2005). IF embedded in a matrix form by KAP, they connected together by a disulfide bond of cysteine residues to form the hair shaft.

Gene expression was a process that gene produce biologically active protein through transcription and translation. The study on gene expression difference was one of the most promising methods. Transcription was an important part of gene expression regulation, and mRNA was the target product of transcription. Therefore, the determination of mRNA

expression levels could reflect the level of gene expression and the strength of biological function well (Li, 2004). There was little research about the expression patterns of KAPs family genes in different growth stages on rabbit currently.

As one of the local excellent meat-rabbit varieties in China, Fujian Yellow rabbit was listed in National livestock and poultry genetic resources conservation varieties directory in 2006. The advantages of Fujian Yellow rabbit were as follows: extensive adaptability, strong disease resistance, high reproduction rates, high carcass quality, moreover, high medicinal value (Xie et al., 2009). It received much concern of farmers and consumers after many years breeding by rabbit researchers in Fujian Academy of Agricultural Sciences. Fujian Yellow rabbit has short yellow wool fibre, and the colour of adult was deeper than infancy, which had a white zonal

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hair coat from mandible to groin, blue black eye, little head, short thick ear, high spherical hindquarter.

In this paper, we analysed expression regulation of KAP3.1, KAP3.3 gene in different parts of skin on Fujian Yellow rabbit, German Angora rabbit and American Rex rabbit, researched the developmental change, and analysed expression ways, influence of gene expression on the development of hair length. Through above work, we might provide valuable reference for breeding of wool qualitative character on rabbit.

Materials and Methods

Animals and sample collection

The samples in this study were collected from Fujian Yellow rabbit, German Angora rabbit and American Rex rabbit in Jinling Rabbit Farm of Jiangsu Province, who were mated in the same period. Each breed had ten pregnant females. We collected wool fibre from neck, back, buttock and abdominal when the rabbits were 2, 4, 6, 8, 12, 16, 20 weeks old respectively, three males and three females each breed. We collected the skin of back and buttock and kept in -70°C at the same time.

Length measurement

We measured the length of coarse wool and fine wool, resaved ten values finally and then calculating average respectively.

RNA extraction and cDNA synthesis

Total RNA was extracted following the Trizol Reagent instructions (Invitrogen, Carlsbad, CA). RNA quality and quantity were determined electrophoretically by OD260/OD280 nm absorption ratio between 1.8 and 2.0, and were detected by 1.5% agarose gel electrophoresis (Figure 1). Then mixed males' and females' total RNA of the same development stages respectively.

Reverse transcription to cDNA as described by the manufacturer of Takara kit (DRR037A), reaction condition is: 37°C (15 min), 85°C (5 s). And cDNA were stored at -20°C .

Real-time PCR

RT-PCR reaction mixture contained: 10 μL SYBR Premix Ex TapTM II(2 \times), 0.8 μL PCR Forward Primer(10 μM), 0.8 μL

PCR Reverse Primer(10 μM), 0.4 μL ROX Reference Dye II(50 \times), 2 μL cDNA, 6 μL RNase-free dH₂O. Reaction conditions were as follows: 95°C for 1 min, 40 cycles of 95°C for 15 s and 60°C for 34 s, 95°C for 15 s, 60°C for 1 min, 95°C for 15 s, 60°C for 15 s. Primers sequences were designed according to gene sequence of KAP3.1 and KAP3.3 amplified by Wu Tian-wen (Wu T.W., 2011) and the GAPDH was used for housekeeping gene (Table 1). The exam was performed using ABI 7500 real-time PCR system (Applied Biosystems, USA)

Statistical analysis

The relative expression ratio was calculated with the $-\Delta\Delta\text{Ct}$ method (Livak and Schmittgen, 2001). The calculation formula was: $\Delta\Delta\text{Ct} = (\text{Ct}_{\text{tt}} - \text{Ct}_{\text{tk}}) - (\text{Ct}_{\text{ct}} - \text{Ct}_{\text{ck}})$, where Ct_{tt} was the average of target gene in treatment groups, Ct_{tk} was the average of housekeeping gene in treatment groups, Ct_{ct} was the average of target gene in control group, Ct_{ck} was the average of housekeeping gene in control group.

Results and Discussion

Development of wool fibre length

Daily average growth rate of coarse wool and fine wool in German Angora rabbit were 0.473 ± 0.070 mm and

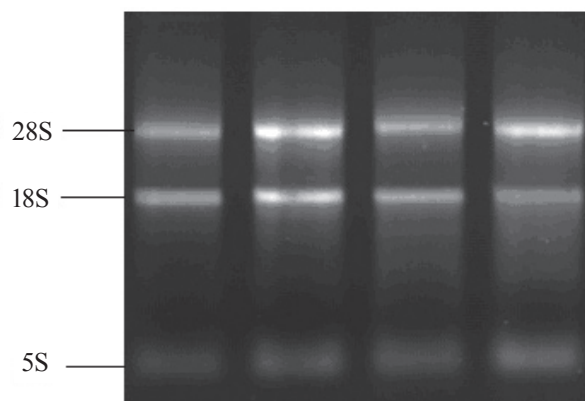


Fig. 1. 1.5% agarose gel electrophoresis of extracted total RNA

Table 1
Primers sequences of PCR amplification

Locus	Primer sequence	Product size, bp	Annealing temperature, $^{\circ}\text{C}$
GAPDH	5'-TCACCATCTTCCAGGAGCGA-3' 5'-CACAAATGCCGAAGTGGTCGT-3'	293	60
KAP3.1	5'-ACCTCTGACAAATGCTGCC-3' 5'-CCAGCAGGATGAGACATAGATT-3'	138	60
KAP3.3	5'-CCCGCCACCACCATCTG-3' 5'-GGAGTTGAGCAGGAAGC-3'	168	60

0.365±0.050 mm respectively, and it was 0.222±0.021 mm and 0.148±0.013 mm in Fujian Yellow rabbit, 0.154±0.013 mm and 0.138±0.013 mm in American Rex rabbit. The results of items analysis showed that the length of wool fibre of German Angora rabbit were the longest in both coarse wool and fine wool, and the Fujian Yellow rabbit took the second place, and American Rex rabbit was the last. Moreover, the length of back and buttock was longer than neck and abdominal; growth rate of wool fibre in American Rex rabbit and Fujian Yellow rabbit were higher than German Angora rabbit before 2 weeks old, while it was opposite after 4 weeks old (Figures 2 to 4).

Analysis on RT-PCR

The expression characteristics of KAP3.1 gene were in Figure 5 when we took expression level of 4 weeks old Fujian Yellow rabbit as internal control. KAP3.1 gene expressed high levels when it was new born, and the levels in Fujian Yellow rabbit and American Rex rabbit were higher than German Angora rabbit. Expression characteristics of different part and growth period on German Angora rabbit were similar except male's buttock which was 4 to 6 weeks old, and the levels were increased slowly with the growth in the main. While there were significant decline on Fujian Yellow rab-

bit and American Rex rabbit when it was 4 and 12 weeks old (Figure 5). The expression characteristics of KAP3.3 gene were similar to KAP3.1 gene (Figure 6).

Discussion

KAPs gene family had been a research focus on the fur field especially in human and sheep. The expressions of KAP6 and KAP8 gene had difference in the vertical direction of skin layer on human and sheep but rat (Shimomura and Ito, 2005). Adelson et al. (2004) studied gene expressed on skin and wool of Merino sheep, they found some keratin gene related to development of hair follicle and hair cycling. Rogers and Schweizer (2005) draw a physical map of KAP4 gene who had 11 family members located in chromosome 17. Meng et al. (1991) found that composition of keratin in Chinese merino wool was similar to Australian merino wool, high-sulphur protein content in wool was higher than Xinjiang fine wool sheep and military reclamation variety fine wool sheep, and genetic diversity of protein concentrated on high-sulphur protein mainly. Wang C.X. et al. (2009) found that genotype had significant effect on wool production and the level of stretch, but no significance on fineness. At present, the studies on KAPs gene family had concentrated on human, sheep but rabbit.

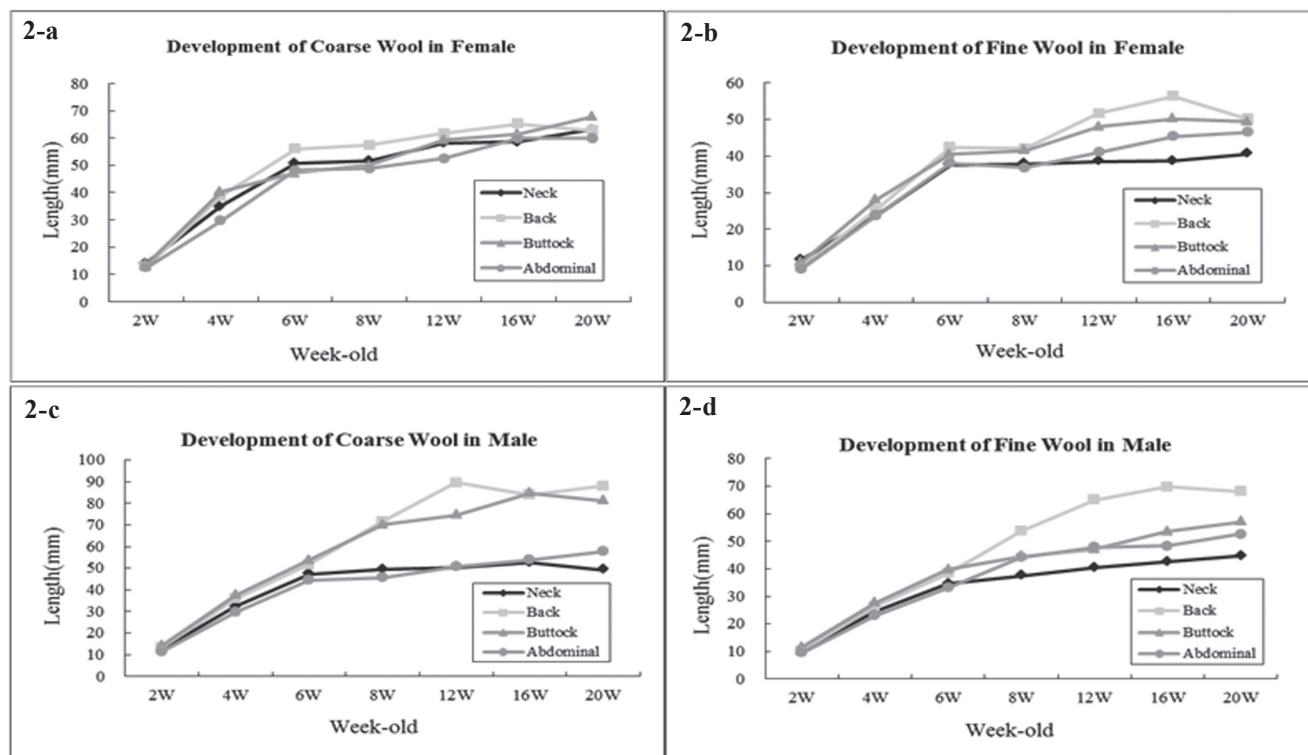


Fig. 2. Development of wool fiber length in German Angora rabbit

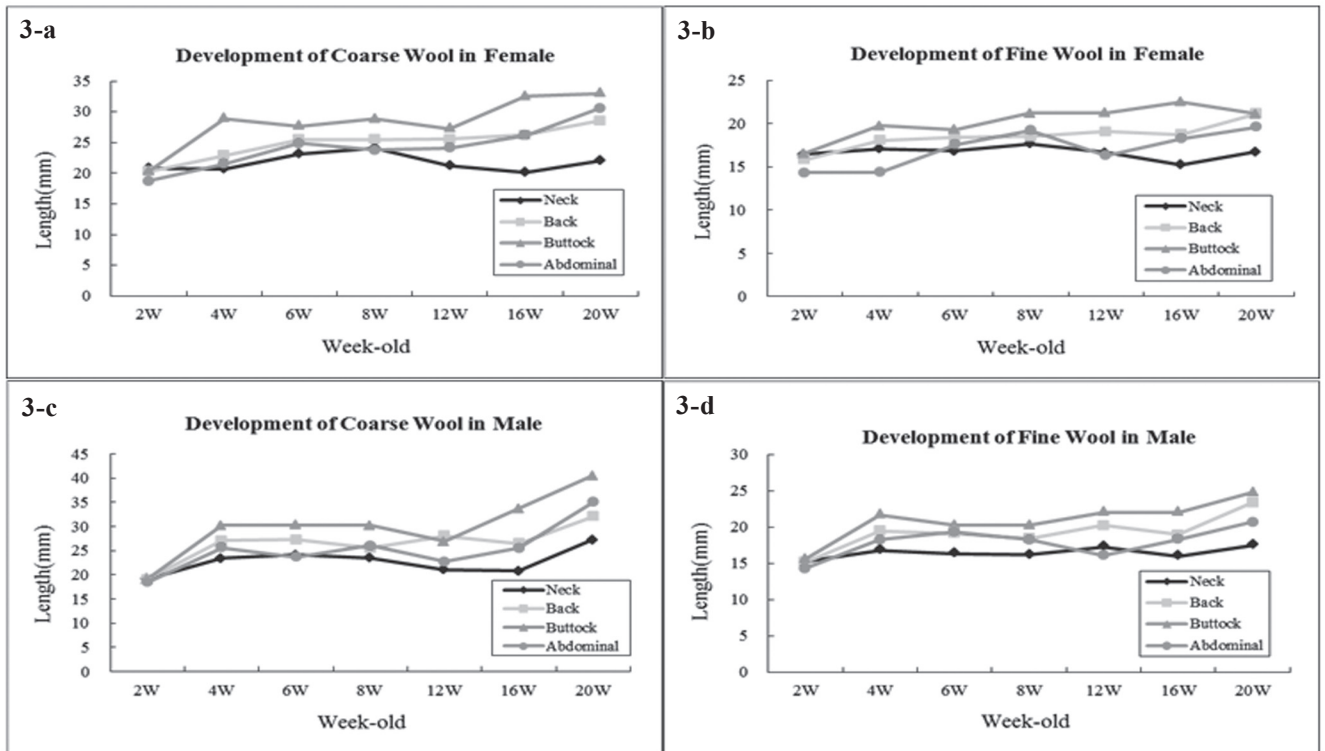


Fig. 3. Development of wool fiber length in Fujian Yellow rabbit

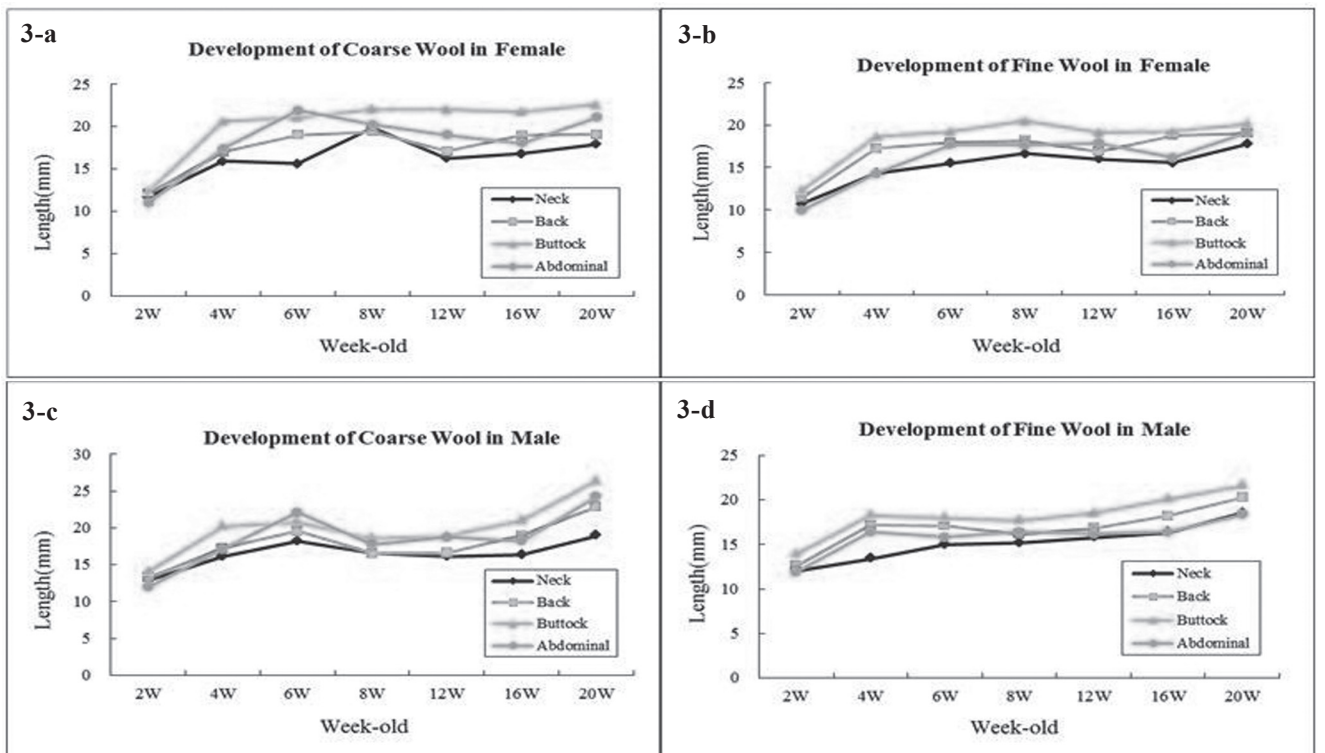


Fig. 4. Development of wool fiber length in American Rex rabbit

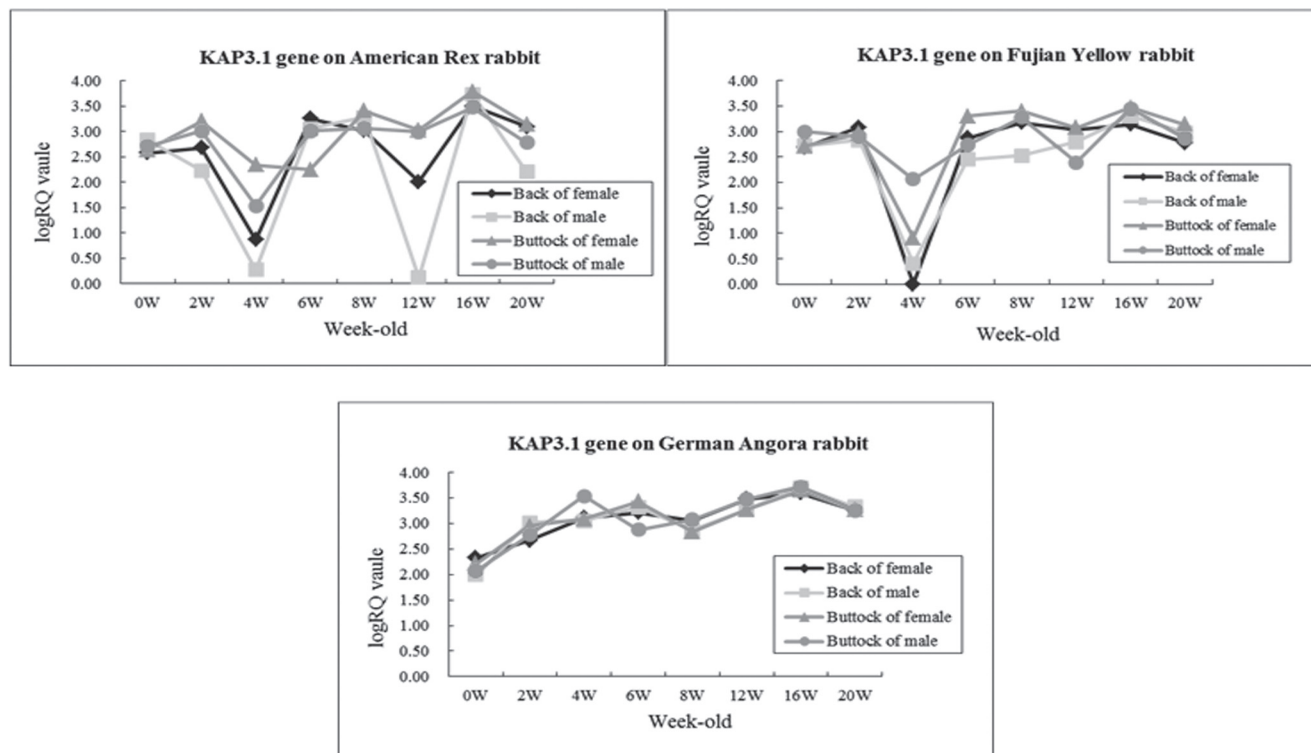


Fig. 5. The comparison of KAP3.1 gene Expression in different rabbit populations of different periods

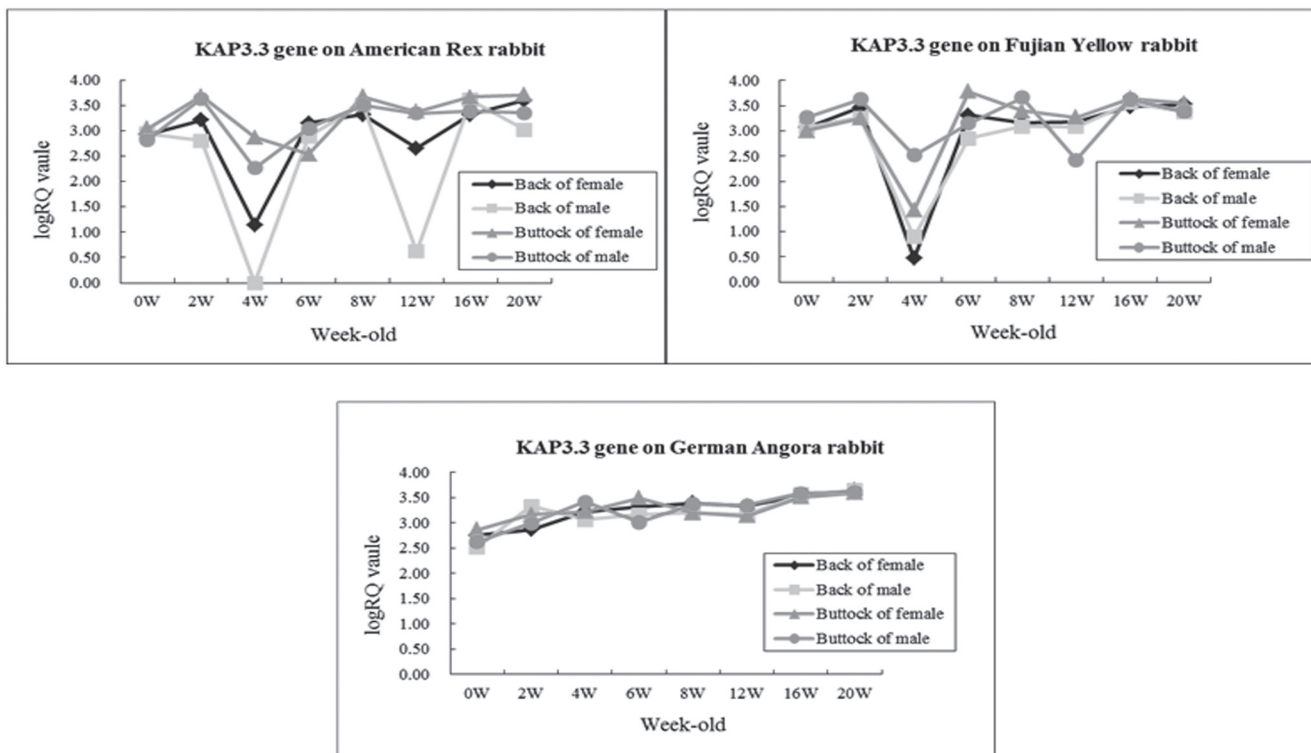


Fig. 6. The comparison of KAP3.3 gene Expression in different rabbit populations of different periods

This study measured the length of wool fiber of different rabbit Varieties in different growth periods. We found that the length of back and buttock were longer than neck and abdominal and coarse wool was longer than fine wool, which was consistent with Li W.H. et al. (2009) and Gu Z.L. et al. (1999). Meanwhile, we analysed expression characteristics of KAP3.1 and KAP3.3 gene by RT-PCR for the first time. The expression characteristic of KAP3.1 gene was basically identical to KAP3.3 gene. Based on this, the writer infers that there were synergistic effect on KAP3.1 and KAP3.3 gene. The growth speed of wool fibre in American Rex rabbit and Fujian Yellow rabbit were higher than German Angora rabbit before 2 weeks old, and it well coincided with high expression level in this period. Moreover, the wool length on German Angora rabbit was longer than the other two varieties, and we found the expression level decreased at 4 weeks old. There might be the correlation between them.

Conclusions

Combined with the development regularity of wool length fibre and the result of RT-PCR, the author speculated that it showed less positive effects after the level of gene expression reducing, and positive effects were not as strong as before even though the expression levels increased later. What is more, expression levels decreased more significantly on American Rex rabbit than Fujian Yellow rabbit when it was 12 weeks old, and the wool length of American Rex rabbit was shorter than Fujian Yellow in general. In a word, we may predict that the length development of wool fiber was associated with KAP3.1 and KAP3.3 genes.

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