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Correlation Analysis Between mRNA Expression of *AMPDI* Gene and IMP Contents in Rabbits

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Abstract: Meat flavor, one of the most important characteristic of meat quality in farm animals is significantly determined by the Inosine Monophosphate Acid (IMP) content. However, the genetic regulation underlying IMP synthesis has largely remained unknown at the present. In this study, researchers cloned the *AMPD1* gene in rabbit and investigated the mRNA expression in longissimus dorsi muscle at 40, 70 and 90 days old, respectively using real-time PCR method. The association between mRNA expression and IMP content was subsequently studied among 48 individuals from two breeds of Tianfu black rabbit (n = 24) and Harbin albino rabbit (n = 24). Total 899 bp fragment containing entire coding region was obtained for rabbit *AMPD1* gene. There is no significant difference of IMP content between the two breeds rabbits in the three studied ages ($p>0.05$). The slaughter age has significant effect on the IMP content with the highest value at 90 days old (3.41±0.29) and the lowest values at 40 days old (2.50±0.39). Researchers detected the negative correlation between IMP content and AMPD1 mRNA expression ($r = -0.916$, $p<0.05$). The *AMPD1* gene have not significant effects on IMP contents. The results suggested the potential function of AMPD1 to determine the IMP content in rabbit which is significant to conduct the MAS for improving the meat quality.

Key words: Meat flavor, IMP, mRNA expression, AMPD1, rabbit, China

INTRODUCTION

During the past decades, more and more attentions have been cast to the improvement of meat quality in farm animals especially for the characteristics of tenderness, carnation and water preserving capability. Meat flavor as one of the most important characteristic of the meat quality has been always ignored in traditional breeding program.

The Inosine Monophosphate acid (IMP) content, playing a major role in umami taste as an umami substance is one of the key factors determining the meat flavor (Berta *et al*., 1990).

It has been wildly suggested that the IMP content is largely determined by the breed, age, sex and muscle tissues in chicken (Davide and Khan, 1967; Khan *et al*., 1968; Chow and Jacobson, 1968). Although, the content of IMP is genetically determined however which is difficult to improve by using traditional breeding methods. In contrast, candidate gene method may be an alternative approach to study these traits presently.

The synthesis of IMP *in vitro* had two pathways, de novo synthesis and salvage synthesis (Aimi *et al*., 1990). The de novo synthesis of the nucleotide IMP is involved 10 kinds of key enzymes. Among them, the Adenosine Monophosphate Deaminase (AMPD) plays a critical role in the regeneration of IMP from ATP via the purine nucleotide cycle (Sabina and Holmes, 1995). The *AMPD1* gene exists in all eukaryote and expressed predominantly in skeletal muscle during muscle development *in vivo* and during myocyte differentiation *in vitro* (Noumi *et al*., 1984; Sabina *et al*., 1989).

So far, the study about AMPD1 is almost concentrated in human and rat. The Single Nucleotide Polymorphisms (SNPs) of *AMPD1* gene have been wildly reported to significantly associate with the susceptibility to facilitating amyotrophy (Abe *et al*., 2000), protecting the heart (Yacoub *et al*., 2004; Yazaki *et al*., 2004), regulating the catabolism of ATP in skeletal muscle (Norman *et al*., 2001), contributing to the high incidence of myoadenylate deaminase deficiency in the caucasian population (Gross *et al*., 2002) and the acetone body

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characteristics of pig (Aimi *et al*., 1990). Few researches were reported on polymorphisms of AMPD1 and its relation with muscle IMP content especially in rabbit.

At present, no study has revealed the influence of *AMPD1* gene to the content of IMP in rabbit. The objective of this study aimed to identify the IMP content in rabbits and analyze the relationship between the expression of *AMPD1* gene and the content of IMP which will be benefit for cattle breeding and genetics.

MATERIALS AND METHODS

Animals and tissue collection: The health Tianfu black rabbits and Harbin albino rabbit (24 individuals for each breed) were collected in this study with similar body weight. Eight rabbits were randomly assigned to each stage for each breed with *ad libitum* access to feed under same normal conditions. The animals were reared according to the national regulations for the humane care and use of animals in research. The rabbits were slaughtered at nursery of Sichuan Agriculture University at 40, 70 and 90 days old. The whole longissimus dorsi muscle was rapidly and manually dissected from each rabbits. These samples were snap frozen in liquid nitrogen and then frozen at -70°C until the samples were subsequently selected for extraction of the total RNA and determination of inosine monophosphate IMP.

Detection the content of IMP: High Performance Liquid Chromatogram (HPLC) was used to determine the Inosine Monophosphate (IMP) in the longissimus dorsa of rabbit according to the manufacturer's instructions and the standard substance is inosine 5'-monophosphate disodium salt.

Total RNA extraction and cDNA synthesis: Total RNA was extracted from longissimus dorsa using RNAprep pure Tissue Kit according to the manufacturer's protocol (TIANGEN, Beijing, China). Reverse transcription was performed in a final volume of 20 μL containing 12 μL of Rnase Free dH_2O, 4 μL of 5×PrimeScript™ Buffer (for Real Time), 1 μL of PrimeScript™ RT Enzyme Mix I, 1 μL of Oligo dT Primer (50 μM), 1 μL of Random 6 mer (100 μM) and 1 μL total RNA. (TaKaRa, Dalian, China) and incubated at 37°C for 15 min and at 85°C for 5 sec. The reaction products were stored at -20°C.

PCR amplification and clone: Total RNA was extracted from longissimus dorsa using RNAprep pure Tissue Kit according to the manufacturer's protocol (TIANGEN, Beijing, China). Complementary PCR primers for the mRNA sequence obtained from the National Center for Biotechnology Information's (NCBI) GenBank were designed using the Primer premier 5.0 software for β-actin and AMPD1 (Table 1). Conditions for amplification consisted in 5 min of initial denaturalization at 94°C followed by 37 PCR cycles (30 sec at 94°C, 34 sec at 58°C). The RT-PCR products were gel purified, extracted using the agarose gel extraction kit and cloned into the pGEM-T vector and then introduce the resultant plasmids into E. coZi JM109 competent cells. The recombinant plasmids were identified by PCR and sequenced (Invitrogen, Shanghai, China).

FQ RT-PCR: One-step SYBR Green I-based RT-PCR amplification was performed in the Cycler iQTM PCR system. All PCR reactions were carried out with the following PCR conditions: 10 sec at 95°C followed by 45 cycles of 10 sec at 95°C, 30 sec at 64°C, 1 min at 95°C and 1 min at 55°C with optics turned on. For SYBR Green I-based RT-PCR amplification, amplification plots and Tm values were routinely analyzed to verify the specificities of the amplicons. The standard curve and the comparative assessment of Ct (the cycle threshold, the number of PCR cycles required for the fluorescent signal to rise above background) from samples run in parallel were used to determine the relative abundance of each gene.

Statistical analysis: Statistical analyses were performed using SPSS version 10.0. The disparity of IMP content between rabbit breeds, developmental trend of IMP content and the effect of IMP content treatment on *AMPD1* gene mRNA expression were calculated using t-test. A value of $p<0.05$ was regarded as significance.

Table 1: The primers ussed to clone *AMPD1* gene and RT-PCR analysis

Purposes	Genes/Fragments	Primers	Length (bp)	Anncaling temperature (°C)
To clone *AMPD1* gene	Primer 1	F-GTTCATGCTGGACGCCAGAC;	600	58
	-	R-TTCATGCCTCGTTCCTTTCTC	-	-
	Primer 2	F-CTTATACTTACTATGCCTACT;	633	60
	-	R-TTTATTCATTCTGTTGCTT	-	-
RT-PCR	Primer 3	F-GGAGGTAGGTGCAGACTTGG;	134	64
	-	R-TGTTAGAGCTGTAGATGCGGTT	-	-
	β-actin	F-AGAGCTATGAGCTGCCTGAC;	375	64
	-	R-TCGTACTCCTGCTTGCTGA	-	-

RESULTS AND DISCUSSION

In this study, the researchers obtained the *AMPD1* gene of 899 bp fragment which was deposited in Genbank (Accession No. GQ141053). To date mostly researchs about IMP were execute in chickens and pigs, very few were in rabbit. This is the first report to study the IMP content between breeds of rabbits and analyze the developmental trend of IMP content. In this study, the content of IMP assessment was shown (Table 2). There are no significantly difference of IMP content between two breeds of rabbits ($p>0.5$) and the IMP contents gradually decrease along with growth, the highest contents of IMP was represented at 90 days old (3.41±0.29) and the lowest one was at 40 days old (2.50±0.39). In some chicken breed, the IMP content of muscles of individual was different (Chen *et al*., 2000). The IMP content of beijing fatty chickens had significantly higher than that of white chickens with AA genotype (Chai *et al*., 2005). One recent report studied the IMP content of different part of Duroc in different breeds and found it is not significant deviation among them (Guo *et al*., 2009). The method of determination, the size of animal and breeds of animal could be responsible for this difference. Some early reports have found that he IMP contents gradually decrease along with growth which is the same as us (Li *et al*., 2003). In contrast, the other ones (Chen *et al*., 2002; Shu *et al*., 1989) have the opposite results. The comparatively individual differences of laboratory animals may be the most probable reason for this condition.

In this study, the expression of the *AMPD1* gene in the longissimus muscle of rabbits was determine, the results were shown (Table 3). The highest expression of the *AMPD1* gene was appeared at 40 days old (1.04±0.16) and the lowest ones was at (0.52±0.11). The quantitative relationship between expression of *AMPD1* gene and the IMP content was revealed. There was significant negative correlation effect on IMP content ability evaluated by expression of AMPD1 ($r = -0.916$, $p<0.05$). In the past decade, the researches about the candidate genes or genetic markers related to muscle IMP content are few. AMPD1 as a critical role in the regeneration of IMP from ATP via the purine nucleotide cycle has been studied in this years (Sabina and Holmes, 1995). One early report analyzed the sequence diversity of *AMPD1* gene and its relationship with IMP content in various chicken breeds, two polymorphism sites A120G and A355G were deduced may have significant effects on IMP contents (Zhang *et al*., 2004). Another report analyzed a 500 bp fragment of *AMPD1* gene using PCR-SSCP method and analysis of variance indicated that this AMPD1 genotypes did not significantly influence the IMP content in chicken (Chai *et al*., 2005) but the opinions have not been unified. According to the synthesis of IMP *in vitro*, researchers could suppose that *AMPD1* gene is not the major gene to affect the IMP content and AMPD1 collaborative other enzymes to participate the synthesis of IMP in rabbit.

To the knowledge, this is the fist report to clone a part of rabbit nucleotide sequence of rabbits AMPD1 mRNA, study the difference of IMP content among ages and breeds in rabbit and reveal the quantitative relationship between expression of *AMPD1* gene and the IMP content.

Table 2: Contents of IMP in ongissimus dorsi muscle of in different breeds and days old rabbits

Groups	Day old	Tianfu black rabbits	Harbin albino rabbit
A	40	$2.55±0.37^{Aa}$	$2.45±0.40^{Aa}$
B	70	$2.90±0.41^{Aa}$	$2.66±0.41^{Aa}$
C	90	$3.46±0.28^{Bb}$	$3.36±0.33^{Bb}$

Means within rows with different superscripts differ significantly ($p<0.05$), different capital letters means highly statistically significant ($p<0.01$)

Table 3: The relative AMPD1 mRNA levels in different breeds and day olds rabbits

Groups	Day old	Tianfu black rabbits	Harbin albino rabbit
A	40	$1.09±0.23^{Aa}$	1^{Aa}
B	70	$0.99±0.21^{ABa}$	$0.88±0.27^{ABa}$
C	90	$0.52±0.09^{Bb}$	$0.51±0.11^{Bba}$

[a]Means within rows with different superscripts differ significantly ($p<0.05$), different capital letters means highly statistically significant ($p<0.01$); [b]Control group is the average expression of AMPD1 mRNA in 40 days old Harbin white rabbit

CONCLUSION

This study shows that there are no significant difference of IMP content between two breeds rabbits in the three studied ages and the IMP contents gradually decrease along with growth. There is also proof that the *AMPD1* gene have not significant effects on IMP contents.

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