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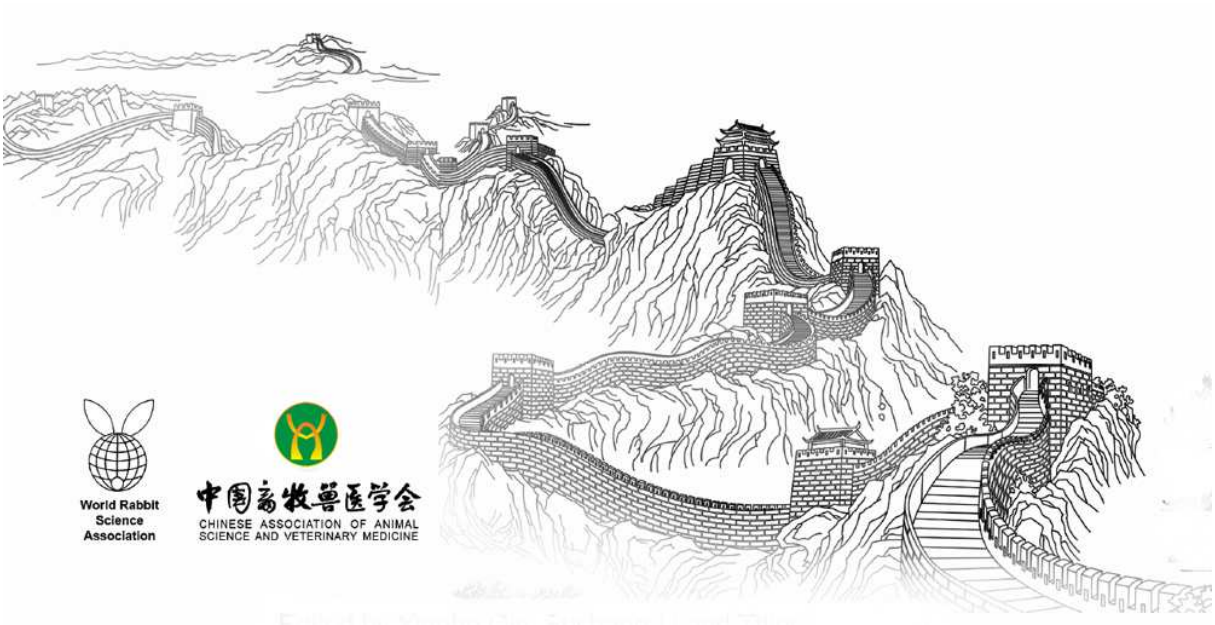

CALPASTATIN GENE POLYMORPHISM IS ASSOCIATED WITH RABBIT MEAT QUALITY TRAITS

Wang J.[1], Elzo M.A.[2], Jia X. [1], Chen S. [1], Lai S. [1]*

[1]College of Animal Science and Technology, Sichuan Agricultural University, Huimin Road 211, 611130, Chengdu, China
[2] Department of Animal Science, University of Florida, PO Box 110910, 32611, Florida, USA
*Corresponding author: Lai S.J. laisj5794@163.com

ABSTRACT

The objective of this study was to investigate single nucleotide polymorphisms (SNPs) in the *calpastatin* (*CAST*) gene and to test their association with meat quality traits in Hyla, Champagne and Tianfu Black rabbit breeds. We detected one SNP located at 67 bp in intron 3 (*CAST*-intron3-T67C) from the set of animals used in this study. The three rabbit populations had intermediate levels of genetic diversity in the *CAST* gene. The statistical analysis indicated that rabbits with the TT genotype had a significantly greater b^*_{0h} and b^*_{24h} than those with the CC genotype ($P<0.05$) in the *longissimus dorsi* muscle. Rabbits with CC genotype had higher intramuscular fat (IMF) than those with CT and TT genotypes in both *longissimus dorsi* and *biceps femoris* muscles in the three breeds ($P<0.05$). Our results indicated that the polymorphism in the *CAST* gene may be used for marker-assisted selection in rabbit meat breeding programs.

Key words: Rabbit, CAST, SNP, Meat quality.

INTRODUCTION

Calpastatins are rich in proline and glutamate but poor in aromatic amino acids. Koohmaraie et al. (2002) showed that the rate of protein degradation postmortem affected the meat quality. Variation in calpastatin abundance influences postmortem aging rates in different muscles (Geesink et al. 1992). Recently, some *CAST* SNPs have been used as commercial genetic markers by livestock industries. Calvo et al. (2014) showed that a new SNP in the *calpastatin* gene was associated with beef tenderness. Cafe et al. (2014) provided further evidence that selection based on *CAST* gene markers may improve meat tenderness in Brahman cattle. The *CAST* gene has a large effect on pork quality (Rohrer et al. 2012) and muscle fiber traits in chicken (Zhang et al. 2012). However, there has been little research on the association between SNPs and rabbit meat quality traits.

The aim of this study was to evaluate the association between the *CAST*-intron3-T67C polymorphism with meat quality traits in the Hyla, Champagne, and Tianfu Black rabbit breeds.

MATERIALS AND METHODS

Animals and meat quality traits

A total of 372 rabbits were used in this study, including 138 Hyla, 139 Champagne and 88 Tianfu Black. Rabbits were reared in individual cages after weaning at 6 weeks of age and fed ad libitum with a commercial diet using standard feeding and management protocols. Rabbits were slaughtered at 70 days of age and samples of ear tissue were collected for DNA extraction.. After slaughter, we measured the pH ($pH_{0\ h}$) and color ($L^*_{0\ h}$, $a^*_{0\ h}$, $b^*_{0\ h}$) within 15 min. Then, the carcasses were stored at 4℃, after chilled for 24 h we measured the pH ($pH_{24\ h}$), color ($L^*_{24\ h}$, $a^*_{24\ h}$, $b^*_{24\ h}$), and intramuscular fat (IMF). Measures were performed in the *longissimus dorsi* and *biceps femoris* muscles.

Detection of SNP and genotyping

Genomic DNA was extracted using AxyPrep Genomic DNA Miniprep Kit (Axygen, USA) and stored at -20℃. PCR primers for the *CAST* gene were designed by the Primer Premier 5 software based on the rabbit gene sequence (Ensembl accession NO. ENSOCUG00000007802) to amplify a 655 bp DNA fragment . The PCR primers for the *CAST* gene were as follows: CAST-F: CATTAGGCCGTTCCAATCAGC, CAST-R:

CCTATGTAGCAGCCCGGTTATTC. The PCR products were directly sequenced on a 3700 DNA sequencer in both directions using the BigDye Terminator sequencing kit (Applied Biosystems, Foster City, CA, USA). Lastly, the sequences were analyzed with DNAMAN software (version 5.2.2).

Statistical Analysis

The genotype and allele frequencies in all breeds were calculated by standard procedures. Hardy-Weinberg equilibrium (HWE) was tested using a likelihood ratio test with software POPGENE (Ver. 3.2). Population genetic indexes including gene heterozygosity (He), effective allele numbers (Ne) and polymorphism information content (PIC) were estimated using the following formulas (Botstein et al. 1980):

$$, \quad He = 1 - \sum_{i=1}^{n} P_i^2 \quad , \quad Ne = 1 / \sum_{i=1}^{n} P_i^2 \text{ and } PIC = 1 - \sum_{i=1}^{m} P_i^2 - \sum_{i=1}^{m-1} \sum_{j=i+1}^{m} 2P_i^2 P_j^2$$

Where P_i was the frequency of the i^{th} allele and n was the number of alleles.

The meat quality traits were analyzed using the following linear model: $Yijk = \mu + G_i + S_j + e_{ijk}$. Where *Yijk* was a meat quality trait, μ was the overall mean for each trait, G_i was the genotype effect, S_j was the fixed sex effect, and e_{ijk} was the random residual. Least squares means and their standard errors were computed for all genotype effects, and pairwise comparisons among them were made using Bonferroni t-tests. Computations were carried out using the general linear model (GLM) procedure of SPSS 21 (IBM, Armonk, NY, USA).

RESULTS AND DISCUSSION

We detected one SNP located at 67 bp in intron 3 of the *CAST* gene. The genotyping of the *CAST*-intron3-T67C polymorphism was successfully implemented using PCR and DNA sequencing. Figure 1 shows a sequencing map of the novel *CAST*-intron3-T67C SNP in the Hyla, Champagne, and Tianfu Black rabbit breeds.. The genotype and allele frequencies, Chi-square test, heterozygosity (He), effective number of allele (Ne), and polymorphism information content (PIC) were calculated and summarized in Table 1. The T allele showed a high prevalence in these breeds.

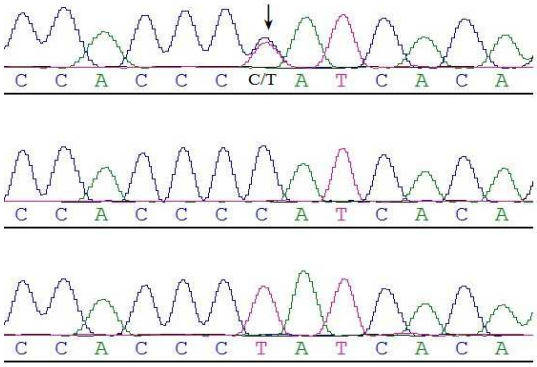

Figure 1: Sequencing map of the novel *CAST*-intron3-T67C in three rabbit breeds

Least squares means in Table 2 showed that rabbits with the TT genotype had a significantly greater b^*_{0h} and b^*_{24h} than rabbits with the CC genotype ($P<0.05$), but similar values for pH (0h, 24h), L* (0h, 24h), and a* (0h, 24h) in the *longissimus dorsi* muscle. No significant differences among the three genotypes were found for pH (0h, 24h), L* (0h, 24h), a* (0h, 24h) and b* (0h, 24h) in *biceps femoris* muscle (Table 3; $P>0.05$). Rabbits with CC genotype had higher IMF than rabbits with CT and CC genotypes in both *longissimus dorsi* and *biceps femoris* muscles among these three rabbit breeds (Tables 2 and 3; $P<0.05$).

Table 1: Diversity parameter for the *CAST* gene in rabbits

Breed (number)	Genotype/number/GF[a]			Allele/AF[b]		χ^2 (HWE)[c]	He[d]	Ne[e]	PIC[f]
Hyla (138)	CC/8/0.0580	TT/80/0.5797	CT/50/0.3623	C/0.2391	T/0.7609	0.3639 $P>0.05$	0.3639	1.5721	0.2977
Champagne (139)	18/0.1295	52/0.3741	69/0.4964	0. 3777	0. 6223	0.4701/ $P>0.05$	0.4701	1.8871	0.3596
Tianfu Black (88)	14/0.1591	40/0.4545	34/0.3864	0. 3523	0. 6477	0.4564/ $P>0.05$	0.4564	1.8394	0.3522
Total rabbit (365)	40/0.1096	172/0.4712	153/0.4192	0. 3192	0.6808	0.4346/ $P>0.05$	0.4346	1.7687	0.3402

[a] GF: Genotypic frequency. [b] Allelic frequency. [c] $\chi 2$ (HWE): Hardy-Weinberg equilibrium $\chi 2$ value. Hardy-Weinberg equilibrium (P>0.05), Hardy-Weinberg disequilibrium (P<0.05). [d] He: Gene heterozygosity. [e] Ne: Effective allele number. [f] PIC: Polymorphism information content.

Table 2: Least square means for CAST genotype effects on meat pH, color and IMF traits in rabbit *longissimus dorsi* muscle

	CAST Genotype[a]			
Trait[b]	CC	TT	CT	*P*-value
pH_{0h}	6.47±0.08	6.72±0.08	6.32±0.05	0.176
pH_{24h}	5.71±0.03	5.71±0.03	5.74±0.02	0.132
L^*_{0h}	47.70±1.04	49.38±1.03	48.63±0.68	0.304
L^*_{24h}	56.87±0.75	60.23±1.10	58.32±0.71	0.264
a^*_{0h}	4.77±0.41	5.14±0.32	5.67±0.27	0.144
a^*_{24h}	4.02±0.23	4.77±0.44	4.40±0.47	0.137
b^*_{0h}	1.39±0.19[c]	3.09±0.11[a]	2.81±0.13[ab]	0.037
b^*_{24h}	4.62±0.20[c]	5.40±0.22[a]	5.20±0.23[ab]	0.048
IMF (%)	2.52±0.11a	1.49±0.09b	1.73±0.21ab	0.029

Values with different superscripts within the same row differ significantly at $P<0.05$ (a, b) and $P<0.01$ (A, B, C).

In our study, color values were similar to values reported in previous research (María et al. 2006; Trocino et al. 2002). The small differences in color trait values between *longissimus dorsi* and *biceps femoris* muscles here partially agreed with the results reported by Chiericato et al. (1996), who found that yellowness was not consistent across different muscles. The result for IMF indicated that the *CAST*-intron3-T67C SNP maybe useful for rabbit meat breeding. Further work is necessary with larger rabbit populations to elucidate the mechanisms involved in this gene's effect on rabbit meat quality.

Table 3: Least square means for CAST genotype effects on meat pH, color and IMF traits in rabbit *biceps femoris* muscle

	CAST Genotype[a]			
Trait[b]	GG	TT	GT	*P*-value
pH_{0h}	6.58±0.14	6.52±0.26	6.18±0.18	0.226
pH_{24h}	5.73±0.05	5.72±0.04	5.77±0.02	0.125
L^*_{0h}	52.79±0.80	52.70±0.86	52.94±0.77	0.312
L^*_{24h}	60.93±1.01	62.39±0.92	62.32±0.87	0.208
a^*_{0h}	3.32±0.35	4.15±0.21	3.92±0.33	0.114
a^*_{24h}	4.55±0.23	4.89±0.31	4.53±0.33	0.272
b^*_{0h}	2.05±0.16	2.51±0.19	2.69±0.21	0.119
b^*_{24h}	5.52±0.28	5.17±0.21	5.98±0.31	0.071
IMF(%)	3.29±0.31a	1.68±0.13b	2.88±0.23ab	0.038

Values with different superscripts within the same row differ significantly at $P<0.05$ (a, b) and $P<0.01$ (A, B, C).

CONCLUSIONS

Based on our results, it could be concluded that the *CAST*-intron3-T67C polymorphism may be a useful potential marker for meat quality traits in marker-assisted selection programmes in rabbits. This assumes that this SNP maybe closely associated with QTL affecting meat quality traits.

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