Journal of Animal &Plant Sciences, 2013. Vol.20, Issue 2: 3122-3125
Publication date 31/12/2013, **http://www.m.elewa.org/JAPS**; *ISSN 2071-7024*

Discovery of a SNP in exon 2 the lipoprotein lipase gene and its association with intramuscular fat content in Chinese ring-necked pheasant

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Keywords: Chinese ring-necked pheasant (*Phasianus colchicus*), *LPL*, PCR-SSCP, Single nuclotide polymorphism

1 SUMMARY

Lipoprotein lipase (LPL) **plays a major role in the process of enzyme reaction, and is considered as a functional candidate gene for genetic markers in lipid deposition. This study was designed to investigate the effects of variants in exon 2 of *LPL* gene on Chinese ring-necked pheasant intramuscular fat (IMF) content. Primers for exon 2 of LPL gene were designed from chicken genomic and cDNA sequences. Polymorphisms were detected by DNA sequencing, and PCR-SSCP method was used to confirm genotype. The results showed one novel polymorphism, a synonymous alteration in exon 2 of *LPL* gene (c.6731 T>C). It was associated with breast and thigh IMF content of Chinese ring-necked pheasants. The results suggested that alteration in exon 2 of *LPL* gene might be linked with potential major loci or genes affecting IMF content.**

2 INTRODUCTION

Meat quality depends on not only environment and nutrition but also the fatty acid composition. The IMF (intramuscular fat) content has been recognized as an important carcass trait that affects meat quality, such as juiciness and taste. *LPL* gene is assumed to be a major candidate gene for genetic markers in lipid deposition; hence, it has been positively correlated with meat quality. The *LPL* gene is one of the candidate genes for detecting polymorphisms associated with the adipose deposition and metabolism in chicken, pig, cattle and other animals. The Chinese ring-necked pheasant is raised by commercial farms in most parts of China because of special fleshy flavour, such as good fecundity, good adaptability, and has some ferity. However, information on *LPL* gene and its polymorphism in domestic Chinese ring-necked pheasant still remains very scarce. Therefore, this study is to detect the relationship between polymorphism of exon 2 of *LPL* gene and its association with IMF content in Chinese ring-necked pheasant.

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3 METHODOLOGY

The chicken *LPL* gene sequence (NC_006127) was used for primer design (L-F, TGGGTGGACGGTGACCGGA and L-R, AGCAATCCCAGCAGCATG). The PCR programme was as follows：initial denaturation at 94℃ for 5 min, followed by 35 cycles at 94 ℃ for 30 s, annealing at 54℃ for 30 s, and 72℃ for 30 s, with a final extension at 72℃ for 10 min. PCR was performed in a volume of 25μL consisting of about 1.0μL of DNA template (containing about 50 ng DNA), 1.0μL each of forward and reverse primers, 2.5μL 10×PCR buffer, 2.0μL deoxynucleotide triphosphates, 0.25μL Taq DNA polymerase, and 17.25μL H_2O. PCR products were checked in 1% agarose gels stained with ethidium bromide.

A total of 120 male and 120 female Chinese ring-necked pheasants were randomly selected for SNP (????) identification. All birds were reared, weighed, blood sampled, and slaughtered with appropriate humane methods at 150 days of age. Venous blood was collected from each bird the day before slaughter. After exsanguinations, scalding, and defeathering, the breast and thigh muscles were obtained after deboning. The IMF content was extracted using the soxhlet extraction technique. Three PCR fragments were found, homozygotes with different genotypes were selected for direct sequencing. Sequencing was performed by Bio-Engineering Co., Ltd. (Shanghai, China). A DNASTAR package was used to analyze the sequences and to identify polymorphism.. The association between the different genotypes and each polymorphic locus and the IMF content was carried out using the general linear model (GLM) in SAS version 8.12 (SAS Institute, USA).

According to the following model：

$Y=\mu+G+e$,

Y as intramuscular fat content,

μ as the mean of group,

G as effect of genotype,

e as random residual effect.

When a statistical significance was detected ($P<0.05$), comparisons between means were carried out using a multiple range test.

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4 RESULTS AND DISCUSSION

In this study three genotypes were clearly discerned, and were named TT, TC and CC. A novel single nucleotide polymorphism was detected (c.6731 T>C), but the alteration was synonymous and did not result in an amino acid change. There was 0.2879 in polymorphic information content (PIC) of *LPL* gene. CC gene frequency was 0.7083 and C genotype frequency was 0.7750. Association analysis revealed that there were significant differences among homozygote TT and heterozygote TC for breast and thigh IMF content of Chinese ring-necked pheasants ($P<0.05$). There were no significant differences among homozygote CC and heterozygote TC for breast and thigh IMF content of Chinese ring-necked pheasants ($P>0.05$) in the Table 1.

Table 1: Association of *LPL* gene SNP polymorphisms with Chinese ring-necked pheasant IMF content

Genotype	Trait	
	Breast IMF content	Thigh IMF content
TT	1.33 ± 0.06^{a}	2.65 ± 0.08^{a}
TC	1.05 ± 0.03^{b}	2.50 ± 0.09^{b}
CC	1.16 ± 0.06^{b}	2.51 ± 0.04^{b}

* Data with different superscripts with in columns are significantly different ($P<0.05$), data with the same superscripts with in columns are not significantly different ($p>0.05$).

Many studies reported that the *LPL* gene was associated with fatness traits in different species. In the study, the *LPL* gene was selected as a candidate gene to investigate associations of gene polymorphisms with IMF content in Chinese ring-necked pheasant populations. The alteration was strongly associated with the IMF content in Chinese ring-necked pheasants and therefore with lipid metabolism. The study has shown that the PIC indicated medium polymorphism ($0.25<PIC<0.5$) in Chinese ring-necked pheasant *LPL* gene (low polymorphism if PIC value < 0.25, medium polymorphism if 0.25 < PIC value < 0.50, and high polymorphism if PIC value > 0.50). Allele C is higher than the allele T in Chinese ring-necked pheasants, which indicated that the *LPL* gene may be a major candidate gene or linked to a major candidate gene that impacts Chinese ring-necked pheasants lipid metabolism. The SNP can be used in molecular marker-assisted selection as genetic for Chinese ring-necked pheasant fatness traits.

5 ACKNOWLEDGMENTS

This work supported by the Project of the National Basic Condition Platform of Ministry of Science and Technology of the Peoples Republic of China (Grant No: 2005DKA21102).

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