Detection of polymorphism in exon 26 of apoB gene in Khazak breed chickens

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Abstract: The aim of the present study was to detect the polymorphism in exon 26 of apoB gene in Khazak breed Chickens. Apolipoprotein B (apoB) is the primary apolipoprotein of low density lipoproteins, and is responsible for carrying cholesterol to tissues. The apoB gene is located on chromosome 3 and consists of thirty exons and twenty-nine introns. in this study, sixty sample selected of laying chickens native khazak breed to the region of Sistan and Baluchestan and DNA genomic was extracted from follicle end feather and genotyping by the PCR-RFLP method. The PCR product was digested with restriction enzyme *AcyI* and then separated electrophoresis using 2% agars gel. A mutation was identified on exon 26 in chromosome 3. AA, AB and BB Genotypes were detected in this population, respectively with frequency of 16.7, 28.3 and 55 percent. The frequency of an allele and B allele was estimated, respectively 0.3083 and 0.6917 percent. The B allele was more frequent than B allele and BB genotype was more frequent than other genotypes in this population. Chi square(χ^2) test and G test showed Hardy- Weinberg equilibrium in the population and this polymorphism was approved using sequencing method with ABI sequencing system .Thus, according to the results observed and the important role of this gene in egg production, this gene appears to be an important candidate gene in native chickens used in breeding program.
[Amin Shahabi, Hamid Reza Seyedabadi, Zahra Roudbari**, Detection of polymorphism in exon 26 of apoB gene in Khazak breed chickens**. *Life Sci J* 2012; 9 (4):330-333](ISSN:1097-8135). http://www.lifesciencesite.com. 50

Key word: Polymorphism, ApoB gene, Khazak Chickens

Introduction

The selection for rapid growth in meat-type chickens results in an increase in physiological disorders such as obesity. Production performance and fitness traits are negatively correlated in the chicken (Pinard-van der Laan et al., 1998; Alex et al., 2002). To improve production and fitness traits simultaneously, molecular markers associated with one or both sets of traits may be useful. Understanding the genetic control of growth in chickens will provide an opportunity for genetic improvement of production performance and physiology (Li et al., 2003). Apolipoproteins are responsible for the transport of lipids and cholesterol. Apolipoprotein B (apoB) is the primary apolipoprotein of low density lipoproteins, and is responsible for carrying cholesterol to tissues and Apolipoprotein (apo) B is a major protein component of plasma very low-density and low-density lipoproteins (VLDL and LDL, respectively) and serves as a recognition signal for the cellular binding and internalization of LDL by the apoB receptor. In contrast to the situation in mammals, avian apoB is also a component of specialized VLDL particles that are produced by the liver in response to estrogen and it is the frame albumen of fat albumen synthesis exudation and transportation, and it has important function for the energy transportation and metabolism, and it can directly or indirectly influence the fat accumulation and growth (Kirchgessner et al, 1987). The apoB gene is located on chromosome 3 and consists of thirty exons and twenty- nine introns(Todd et al 1987). The apoB albumen in mammals mainly includes two forms, i.e. apoB-100 and apoB-48, and they are coded by same gene, and formed by special compiling mechanism (Richardson et al., 2005). The mRNA of apoB in the chicken small intestine is not be compiled, so the expression of apoB-48 doesn't exist in the chick small intestine(Makoto et al., 1999). Zhang et al (2006) found a T/G synonymy mutation on extron 26 of chicken apoB gene, and it had large influence to the weight and ventral fat traits, and 1 week weight and 3 week weight of GG gene type were significantly lower than other gene type, but the ventral fat weight and ventral fat rate of TT gene type were significantly higher than GT gene type and GG gene type. Therefore the present study was designed to elucidate the apoB gene polymorphisms in exon 26 in Khazak breed Chickens based on PCR-RFLP and sequencing methods.

Material and Methods

Chicken Populations

Khazak breed chicken used in this study. The chicken is a laying breed and are native to the region of Sistan and Baluchestan.

DNA Extraction

Feather samples were collected from sixthy khazak breed chickens. Genomic DNA were extracted using salting-out method with some modifications (Javanrouh et al., 2006). Optimization includes utilization of separate buffer instead of buffy coat isolation, in that chloroform is for DNA phase isolation and is used to purify DNA and sodium acetate for more concentrated DNA. The optimize protocol would be more safe, simple, cheap and rapid.

PCR Amplifications and Genotyping

The apoB primers (5' CAT ATT TCT AAT GGC ATC CAG 3'; 5' TTC CCA GCG TTA TTT CCG 3') were chosen based on the primers design by Zhang et al. (2006) to ampify a 779bp of exon 26 of the apoB gene. Fifteen µl of each PCR reaction contained; 1X PCR buffer; 2mM MgCl2; 0.25µM primers; 200 µM dNTPs; 1 unit of Taq polymerase; 150 ng/reaction genomic DNA and ddH2o. Thermal cycling included initial denaturation at 94^{oC} for 5 min, 35 cycles of 94^{oC} for 1 min, 57^{oC} for 1 min, 72^{oC} for 1 min, and an extension at 72^{oC} for 7 min. A single nucleotide polymorphism (SNP) of the apoB region was detected by digesting 10 µl of the PCR product with *Acy*I restriction endonuclease at 37^{oC} overnight. Restriction patterns were visualized by agarose gel electrophoresis and ethidium bromide staining. Allele and genotype frequencies and their accordance with the Hardy-Weinberg equilibrium were determined by the POPGENE 3.2 software (Yeh *et al*., 1999).

PCR product sequencing

PCR products were purified using ExoSAp-IT kit (USB companies is in Germany) then, were sequencing using primers were complementary fluorescence with primers conected and using of sequencing kit (USB companies is in Germany).the final sequence of Forward and Reverse sequencs was determined and alignment using the software of ABI sequencing system.

Sequences analysis

Sequences analysis were performed using various software. Chromas Life 2.1 was used to view sequences and chromatograms and determine the accuracy nucleotides at each position. Sequences of the three genotypes of Khazak chickens by the MEGA 5 software (Tamura et al 2011) in a file integration and then alignment the sequences obtained, nucleotides were identified substituted, deleted or insert.

Results

The transition of T into G SNP, locating at the 123 base in the exon 26 of the apoB gene creates a restriction site for *Acy*-I endonuclease. The 779-bp fragment was digested with *Acy*-I restriction enzyme. The restriction enzyme *Acy*-I digested PCR producted had fragments of 779 bp for AA homozygotes, fragments of 779, 658 and 121 bp for AB heterozygotes and 658 and 121 bp for BB homozygotes(Figure 1). The genotype and allele frequencies at apoB loci calculated by PopGene 3.2 software (Yeh *et al*., 1999). The B allele was more frequent than A allele and BB genotype was more frequent than other genotypes in this population, χ^2 test ($P<0.05$) showed in this population the genotype distributions were in Hardy-Weinberg equilibrium. (Table 1) and the results of this study are consistent with results seyedabadi *et al* (2010).

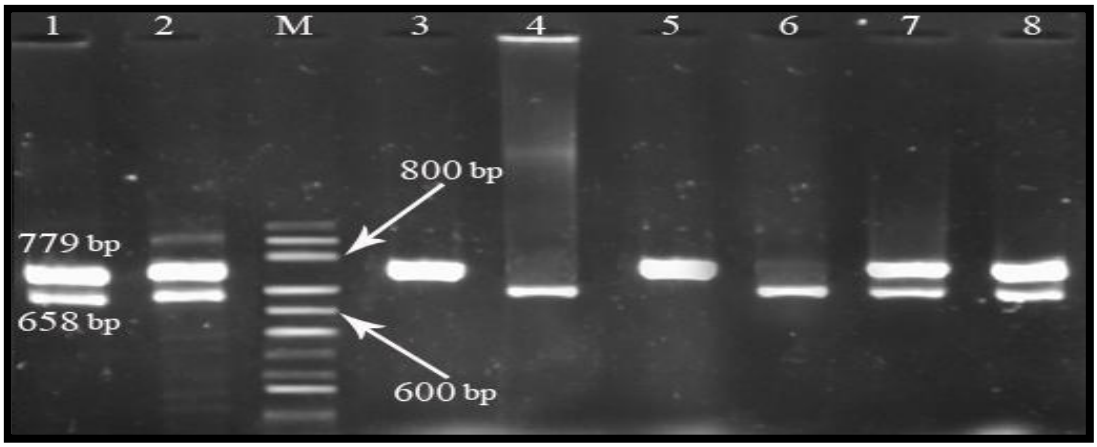

Figure 1- PCR-RFLP pattern for apoB gene with *Acy*-I digestion

Also in this study, tree single nucleotid polymorphism were observed that two of them are type of Transition and one other of them is type Transversion of (Figure 2).

Figure 2- Results of apoB genotypes sequencing

Table 1- Genotype and gene frequency of apoB loci inKhazak chicken

Genotype frequency			Gene frequency		Chi-square test (χ^2)p< 0.05
AA	AB	BB	A	B	
16.7	28.3	55	0.3083	0.6917	0.00

Conclusion

There is allele frequency differences within this population in the present study. Some of these SNP could serve as useful markers for association studies for use in molecular Marker-assisted selection (MAS) programs to control fat deposition. In the study, two new single nucleotide polymorphisms has been found in exon 26 of the Apolipoprotein B gene (apo B) that need further study for finding associated trait in Khazak breed.

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