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Full Length Research Paper

Cytosolic phosphoenolpyruate carboxykinase (PEPCK-C) gene polymorphism in three chicken breeds

Omrani Jalal[1]*, Alipanah Masoud[1] and Hosseini Seyyed Abolfazl[2]

[1]Department of Animal Sciences, University of Zabol, Iran.
[2]Department of Biology, Tarbiat Moallem University of Sabzevar, Iran.

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Phosphoenolpyruate carboxykinase (PEPCK) gene is an enzyme which has a key role in gluconeogenesis. In the chicken genome, there are two different types of PEPCK gene: mitochondrial PEPCK (PEPCK-M) and cytosolic PEPCK (PEPCK-C). In this study, 150 random samples from two native populations in Sistan and Baluchistan Province, namely, Khazak and Dashtiari, and Ross chicken were analyzed. DNA was extracted from feather pulps and PCR was carried out using appropriate primers (F2R2), which amplifies a 1000 bp fragment from the chicken PEPCK-C gene. The fragment covers from promoter to exon 2 of the gene. PCR products were then digested by restriction enzymes *BstEII* and *ACiI*. Allelic as well as genotypic frequencies were determined and analyzed by Popgene software. The allelic frequencies *BstEII* for Khazak sample were A = 0.975 and B = 0.025; for Dashtiari sample; A = 0.95 and B = 0.05 and for Ross samples, A = 0.94 and B = 0.06. The allelic frequencies digestion by *ACiI* for Khazak sample were A = 0.86 and B = 0.14; for Dashtiari sample, A = 0.98 and B = 0.02 and for Ross samples, A = 0.95 and B = 0.05. Haplotype analysis of two single nucleotide polymorphism (SNP) showed that there were four alleles and genotypes. The present results indicated that little polymorphism exists in the samples for this locus.

Key words: Polymorphism, PEPCK gene, Sistan and Baluchistan native chicken, Khazak, Dashtiari.

INTRODUCTION

Phosphoenolpyruate carboxykinase (PEPCK) is an enzyme used in the natural process of gluconeogenesis. It converts oxaloacetate into phosphoenolpyruate and carbon dioxide (Hanson et al.,1997; Cassuto et al., 2005). There are two isozymes encoded by two different nuclear genes (Savon et al., 1993; Jurado et al.,2002), they are PEPCK cytosolic (PEPCK-C) and mitochondrial (PEPCK-M). Sato et al.,1997; Parsanejad et al. (2003) have shown that PEPCK-C, a key regulatory gene in gluconeogenesis, affects RFC. The work showed that the genetic effect of PEPCK-C on RFC was at the level of feed digestion or nutrient absorption.

*Corresponding author. E-mail: Jalal.Omrani@gmail.com. Tel: ++98 5712647682.

Abbreviations: PEPCK, Phosphoenolpyruate carboxykinase; **PEPCK-C,** cytosolic PEPCK-C; **PEPCK-M,** mitochondrial PEPCK; **SNP,** single nucleotide polymorphism.

Sequence analysis of a 2,000-bp fragment of PEPCK-C in White Leghorns revealed the presence of 19 single nucleotide polymorphisms (SNP) that could be grouped into six different haplotype (combinations of SNP alleles). Two of the SNP coincided with RFLP, which enabled us to further subdivide the six haplotype classes into three classes, representing different branches of the gene tree (Parsanejad et al., 2002).

The association of nine PEPCK-C genotypes defined by two RFLP with production traits in a strain of White Leghorn chickens was investigated. The results were normally distributed. Parametric analysis of the traits whose values were close to normal distribution gave the same results. Another study showed that PEPCK-C BstEII only affects the body weight prior to ovulation (HBWT), but not at a later age (MBWT and FBWT) (Muir and Aggrey, 2003).

Analysis of large families showed deviation from Mendelian inheritance of the *PEPCK-C* genotypes, indicating that the PEPCK-C may affect fertility traits

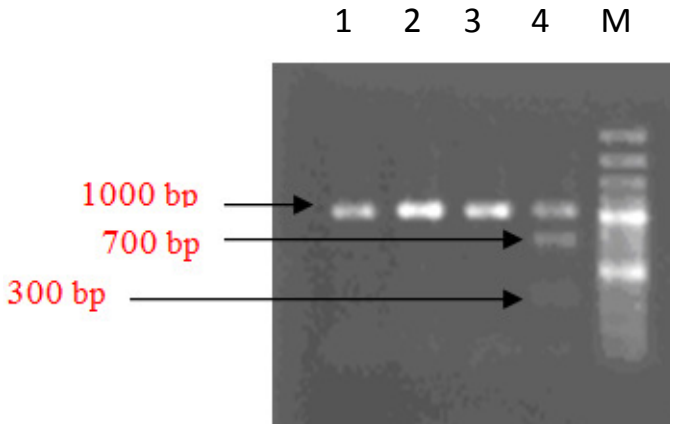

Figure 1. Products of restriction enzyme BSTEII for F2R2 primer. M, 100 bp ladder.

(Parsanejad et al., 2002).

Here, we investigated the polymorphism of PEPCK-C in two native chickens (Dashtiari and Khazak) and Ross chicken by polymerase chain reaction-restriction fragment length polimorphism (PCR-RFLP) method Rabbani, 2008.

MATERIALS AND METHODS

A total of 150 samples of three chicken breeds and 50 samples of each population were genotyped for *PEPCK-M* gene. The chickens were kept in the Institute of Research Domesticated Animals, University of Zabol in the eastern region of Iran. Genomic DNA was isolated from blood samples by the phenol-chloroform method. Amplifications of fragments of PEPCK- C gene were done by PCR. PCR products were amplified using primers as previously described (Parsanejad, 2003): F1R1: forward primer 5'CTGGGACCACCAGCAAGTACTG3, reverse primer 5´GCCTGTGCAGTCGGTGTGTGA´3; F2R2: forward primer 5'GCTGGGACTGAATGGAAGAGGAG3', reverse primer: 5'CTGTTGAGTCGGATGGGTGTCAG3'; F3R3: forward primer: 5´CACCATCAGCTGAAAGGGAGCC3', reverse primer: 5'GTTGGGTTCGTTGGGAGAGACAAC3´; F4R4: forward primer: 5´GTCTCTCCCAACGAACCCAACATG3´, reverse primer 5´CCTCTTCTGACATCCAGCGACC3´.

The PCR was performed in a final volume of 25 µl containing 2 µl of genomic DNA, 0.25 µl of each primer, 0.5 µl of dNTP mixture, 1.5 µl of $MgCl_2$, 0.2 µl of DNA polymerase and 2.5 µl of 10x reaction buffer on an Eppendorf Thermal Cycler. The following PCR protocol was used: initial denaturation at 95°C for 5 min; 35 cycles of 94°C for 60 s, 60°C (primer pair F1R1), or 61°C (primer F2R2) or 58°C (primer pairs F3R3 and F4R4) for 60 s, and 72°C for 90 s; final elongation at 72°C for 8 min.

Individual amplified DNA was separately digested with *BstEII and ACil* enzymes at 37°C overnight. Digestion products were separated electrophoretically in 1.2% (w/v) agarose gel. Frequencies of distribution of alleles were compared with Chi-square test.

RESULTS AND DISCUSSION

Our results show that only F2R2 fragment product had restricted site for *BstEII* enzyme. Here, three genotypes were observed. The BB genotype exhibited 2 bands of 300 and 700 bp, and the AB genotype had 3 bands of 300, 700 and 1000 bp, whereas the AA genotype had a single band of 1000 bp (Figure 1). For *ACil* enzyme, only fragment extended by F4R4 primer pair had restricted site. Digestion by *ACil* produced three genotypes. The BB genotype exhibited single band of 540 bp, and the AB genotype had 2 bands of 540 and 1080 bp, whereas the AA genotype had a single band of 1080 bp (Figure 2).

Tables 1 and 2 show the frequencies of alleles and genotypes within each breed. The genotypic frequencies were calculated by considering presence of various RFLP patterns in three breeds of poultry and are presented in Tables 1 and 2.

Frequency distributions for genotypes and alleles varied among breeds. The A allele in *ACil* or *BstEII* alleles was prominently more frequent than B allele, respectively. Moreover, for *ACil* and *BstEII* genotypes in the breeds, genotype BB were found to be absent in the breeds. Among the breeds, the genotype with the highest frequency was observed in Dashtiari for AA in *ACil* and Khazak for AA in *BstEII* (Tables 1 and 2).

Genotypic frequencies obtained for three breeds of poultry, were tested for equilibrium using chi-square test. The differences among genotypes for all the three varieties were found to be no significant, which indicated that population were in Hardy Weinberg equilibrium. As all populations were in genetic equilibrium, it can be concluded that alleles at this locus have remained free from any factor changing gene and genotypic frequencies.

Based on genotypic frequencies, allelic frequencies were calculated for each variety and are presented in Tables 1 and 2. The allele frequencies were calculated by considering presence or absence of restriction sites at different alleles. The allele A in *ACil* alleles and in *BstEII* alleles were observed in all the three breeds of poultry. The highest allelic frequency 0.98 for A allele in *ACil* and 0.98 in *BstEII* was obtained in Dashtiari and Khazak, respectively.

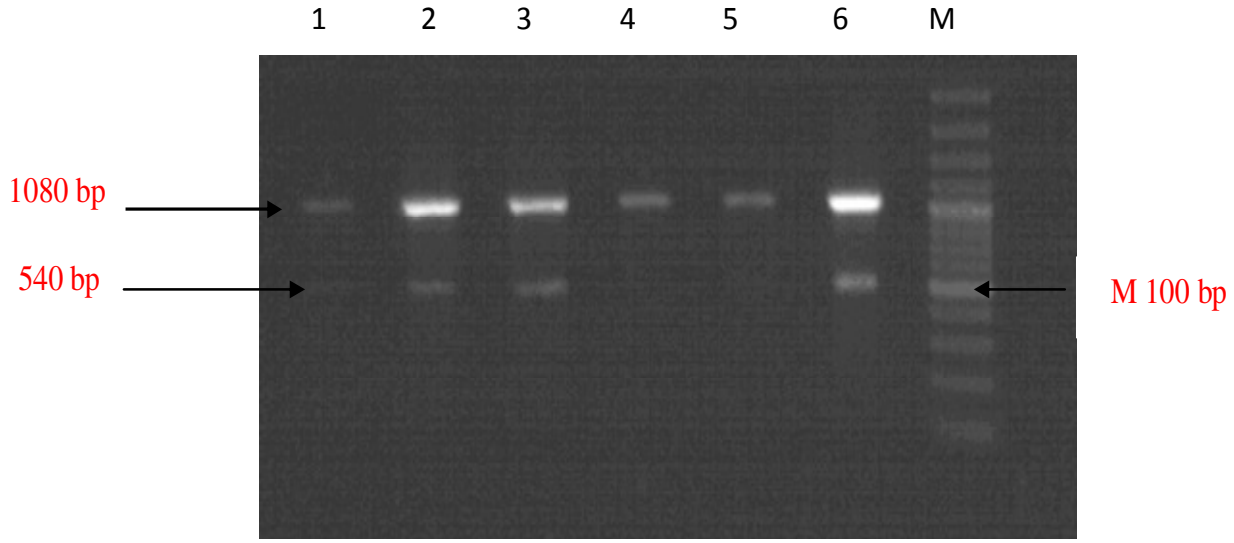

Figure 2. Products of restriction enzyme AciI for F4R4 primer. M, 100 bp ladder.

Table 1. Frequency of genotypes and alleles of PEPCK (*ACiI)* in three chicken breeds.

Chicken breed	Frequency of genotype			Allele frequency	
	AA	AB	BB	A	B
Khazak	0.72	0.28	0.0	0.86	0.14
Dashtiari	0.96	0.04	0.0	0.98	0.02
Ross	0.90	0.10	0.0	0.95	0.05

Table 2. Frequency of genotypes and alleles of PEPCK (*BstEII*) in three chicken breeds.

Chicken breed	Frequency of genotype			Allele frequency	
	AA	AB	BB	A	B
Khazak	0.96	0.04	0.0	0.98	0.02
Dashtiari	0.84	0.16	0.0	0.92	0.08
Ross	0.88	0.12	0.0	0.94	0.06

The RFLP haplotype (*ACiI*–, *Bst*EII–) was diagnostic for C (*ACiI*+, *Bst*EII–), D (*ACiI*+, *Bst*EII+), A (*ACiI*-, *Bst*EII+) and B (Table 3). As expected from assumption that each mutation occurred only once in evolution (one of the four possible combinations of the alleles at two loci is missing), all genotypes could be explained by the presence of the three RFLP haplotypes indicated above. Other researchers showed that there is a restriction cite for ACiI enzyme in Yazd native chickens (Emamgholi et al., 2010). Genotypes diagnostic for the fourth possible haplotype, (*ACiI*+, *Bst*EII+) in two native breeds, Khazak and Dashtiari were absent. Linkage disequilibrium analysis indicates that this conclusion is statistically significant.

The results of haplotype analysis were not more informative than those of single SNP except for body weight trait. The present results indicated that little polymorphism exists in samples of this locus. This result

Table 3. Haplotype frequencies in three chicken breeds.

Haplotype	RFLP alleles		Haplotype frequencies		
	BstEII	***ACiI***	**Khazak**	**Dashtiari**	**Ross**
B	+	-	0.14	0.02	0.06
C	-	-	0.84	0.90	0.82
D	-	+	0.02	0.08	0.08
A	+	+	0.00	0.00	0.04

implies that more information content needs to be provided for further study.

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