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Detection of polymorphism of Insulin-like growth factor-I (IGF-I) gene in native Aseel chicken breed of Pakistan using PCR-RFLP

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ABSTRACT

Molecular analysis is an easier means to identify desirable genotypes for growth. Candidate gene (s) for growth trait like insulin like growth factor (IGF) has imperative function for growth, body composition, fat deposition, metabolic and skeletal traits and the molecular genetic selection on individual genes is a very efficient method to genetically improve economically important traits in chickens. In the present study, polymorphism of the promoter and 5' untranslated region of IGF-I gene of nativeAseel, was investigated. In order to evaluate the IGF-I gene polymorphism, we used the polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method. Feather samples were collected at random from 50 Aseel chickensof Pakistan. Genomic DNA was extracted using modified salting-out PCI (Phenol: Chlorophorm: Iso-amylacohol) method and amplified by polymerase chain reaction. The promoter and 5' untranslated region of the IGF-I gene was amplified to produce a 621 bp fragment. The PCR products were electrophoresed on 2.5% agarose gel and stained by ethidium bromide and then finally on confirmation of amplification the ampliconswere digested Hinf-I restriction enzyme and this revealed two alleles A and B having all three combinations of genotypes i.e. AA, AB were found. Data were analyzed using Pop Gene 3.1 software package. Allele frequencies (A and B) were 0.53 and 0.47 while genotypic frequency fo AB (66) was significantly higher than AA (20) and AB (66). The Chi-

square (χ2) test did not show any deviation from Hardy–Weinberg equilibrium.

1. Introduction

Native poultry breeds such as Desi, Aseel and Naked Neck are poor egg and growth performers but are well adapted to local environment and pathogenesis. In order to make native poultry commercially viable both genetic and nutritional interventions are required. Detection of molecular markers for selection of superior poultry stock for economically important traits and the incorporation of this information into breeding plans for improving native poultry performance offers means to incorporate genetic superiority for higher gains for egg and meat productivity(Zhou et al., 2005; Nishibori et al., 2006).Insulin-like growth factors (IGF1 & IGF2) are mitogenic polypeptides having structural and functional similarity to insulin which play major roles for cellular growth by mediating actions of growth hormone and by affecting a wide range of biological processes, ranging from growth and differentiation to reproduction in poultry (Lei et al., 2005; Zhou et al., 2005; Tang et al., 2010).Studies have found that there is no direct dependence of growth rate in chickens with the levels of growth hormone (GH) therefore it might become useful to study insulin-like growth factors (Beccavin et al., 2001), as mediators of the functions of the growth hormone(Lei et al., 2005; Kadlec et al., 2011).As a result, the chicken IGFs are considered to be the most important candidate genes that can influence chicken performance traits including growth, body measurement, carcass and reproduction. Extensive studies have concluded that IGF-1 is associated with body weight, carcass traits and reproduction traits in chicken.Keeping in view the importance of IGF-I this study was planned to investigate the polymorphisms of the insulin-like growth factor-I (IGF-I) gene in native Aseel breed of Pakistan using polymerase chain reaction - restriction fragment length polymorphism (PCR-RFLP) method.

2. Materials and methods

2.1. Source of experimental material

Experimental birds used in this study were procured froma resource population maintained at “Indigenous Chicken Genetic Resource Centre, Department of Poultry Production, C-Block, Ravi Campus,Pattoki”. Genetic analysis was carried out at “Animal Genetics Laboratory” Department of Livestock production, University of Veterinary and Animal Sciences, Ravi Campus, Pattoki.

2.2. Genomic DNA extraction, qualification and quantification

Genomic DNA was extracted from growing feather pulp using standard Phenol Chloroform Isoamylalcohol (PCI) method (Sambrook et al., 1989). Genomic DNA was qualified and quantified by using Gel-electrophoresis method. All extracted gDNA was stored at -20ºC.

2.3. PCR-RFLP for IGF-I gene

Amplicons with length of 621bpwere digested at 37 °C for an overnight period with 10 U of Pst-I restriction enzyme. After digestion the restriction digests were electrophoresed for 4 hours at 80 Volts on a 2.5% agarose gel with ethidium bromide. PCR-RFLP fragment sizes in each sample were quantified and qualified by using a standard DNA molecular weight marker, by viewing the banding pattern under UV light on the transiluminator.

2.4. Data analysis

The obtained data were statistically analyzed through Pop Gene 1.32 software package (Yeh et al., 1999) to calculate genotypic, allelic frequencies and to check the state of populations about Hardy-weinberg equilibrium.

3. Results and discussions

The digestion of all samples showed that the Aseelchickens were polymorph for IGF-I gene. The PCR-RFLP analysis of gDNA of Desi chicken showed three types of genotypes, named AA (621), AB (621+364+257) and BB (364+257) at SNP (IGF-1-*Pst*1) within 5' flanking section of IGF-1 gene. The genotypic and allelic frequencies of IGF-1 in Desi chicken are given in the Table. 1.

Table 1
The genotypic and allelic frequencies of IGF-1 in Desi chicken.

Chicken	No. of birds	Chi-square test	Average Heterozygosity	Gene frequency		Genotypic frequency		
				A	B	AA	BB	AB
Aseel	50	4.95	0.496	0.53	0.47	20(10)*	66(33)	14(7)

*figures in the blanket are the number of individuals.

The highest observed genotypic frequency was heterozygous AB (66 %) followed by 20 and 14 percent of AA and BB genotypes. As these birds were selected from random mating flocks, never subjected to any kind of selection program, they behaved according to wild populations. Results of this study were consistent with the results of (Wang et al., 2004) who found higher frequency of allele A and genotype AB in chinese native breeds. As the table shows that the frequency of allele A (0.53) than allele B (0.47) so, these results are consistent to (Seo et al., 2001)who found higher frequency of allele A in Korean native ogol chicken. The higher frequency of allele A in Aseel can also be justified with the findings of Tang et al., (2010) who stated that the population carrying higher frequency of allele A showed significantly higher body weight as compared to those who carried lower frequency of allele A, as is the case with Aseel, known for good meat production as compared to Naked Neck and Desi who carried lower frequencies and are low producers as well.

The χ^2(Chi-square) test confirmed that the genotypic frequencies are in coordination with Hardy–Weinberg equilibrium in this population ($p<0.05$). The (Nei, 1973) expected Heterozygosity for IGF-1 in Aseel was 0.49. Result indicates that the Aseel chickens of Pakistan are polymorph to IGF-1 gene.

4. Conclusion

Restriction fragment length polymorphism analysis of this study revealed a SNP (single nucleotide polymorphism) within amplified fragment. So, in the context of importance of IGF-1, its polymorphism can be related to some traits of economic importance i.e. body weight, abdominal fat, egg production.

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