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Investigation of Growth Hormone Gene Polymorphism Using PCR-RFLP Technique in Native Poultry in Khouzestan Province

Darabi Alireza, Fayazi Jamal, Roshanfekr Hedayatolla and Nasiri Mohammad Taghi
Department of Animal Breeding and Genetic,
Ramin University of Agriculture and Natural Resources, Ahwaz, Iran

Abstract: Native animal and poultry in every province are valuable genetic resourses. Khouzestan province native poultry is one of the resourses that bacuase of adaptating to environment. The growth hormone gene is a candidate genes controling the metabolic and physiologic actions. Polymorphism within this gene is a detectable parameter. In order to study and investigate growth hormone gene polymorphism, blood samples were collected from 100 individuals of native poultry and genomic DNA was extracted using salting out technique. DNA fragment with 776 bp at growth hormone gene was amplified using Polymerase Chain Reaction (PCR). The PCR product was digested with restriction enzymes MspI and subsequently electroforesed on 2.5% agarose gel with etidium bromide. The studied intron in this research was at Hardy-Weinberg disequilibrium ($p<0.005$). In this population, heterozygosity and effective allele were estimated 0.59 and 2.13, respectively. Obtained genotypes frequency of MspI enzyme on 776 bp fragment for AA, AB and BB genotypes were 0.39, 0.59 and 0.02, respectively. The frequency of A and B alleles were 0.685 and 0.315, respectively.

Key words: Growth hormone, heterozygosity, native chicken, polymorphism, gene, genotype

INTRODUCTION

In breeding program marker is difference in DNA nucleotides sequence that has mendelian heritance. These differences have participitation in variation between animal significantly and subsequently may be relation between special part of gene that offsprings are received of parents with offspring performance, then we can select offspring by chromosomal part (Nie *et al*., 2002). Growth hormone gene has 4098 base pairs with 5 exons and 4 introns in poultry (Lechniak *et al*., 1999).

It located on chromosome number 19 in avian. The growth hormone is a candidate gene for productive traits, because it has great effect on physiological parameter (Stephen *et al*., 2001).

Kuhnlein *et al*. (1997) were reported that DNA polymorphism in the chicken growth hormone gene has positive correlation with resistance and egg production.

This study is done in other to investigate polymorphism of intron 1 of growth hormone gene. This gene previously used by Zhang *et al*. (1993) and Yao *et al*. (1996) on cattle and Tanaka *et al*. (1992) and Nie *et al*. (2005) on poultry.

MATERIALS AND METHODS

Hundred Blood samples of Khouzestan native chickens were randomly collected from the wing vein using EDTA as an anti-coagulating agent. Blood samples were stored at -20°C.

DNA was extracted from the whole blood using optimized and modified salting-out method (Stephen *et al*., 2001). DNA was quantified spectophotometrically and electrophoresed. Genomic DNA (1 μL) was amplified with 0.3 μL Taq polymerase, 0.5 μL $mgcl_2$, 0.5 μL dNTPs and 2 μL of each primer in a total volume of 25 μL.

The reaction mixture was subjected to an initial 5 min denaturation at 94°C, followed by 35 cycles of denaturation at 94°C for 30 sec, annealing at 60°C for 120 sec, extension at 72°C for 1:30 and a final extension step at 72°C for 3 min.

After the addition of 10 μL of amplification products were loaded on to 1/5% denaturing agarose gels. To visualize the PCR product, gels were stained using etidium bromide. The stained gels were scanned and genotypes were scored.

Corresponding Author: Darabi Alireza, Department of Animal Breeding and Genetic,
Ramin University of Agriculture and Natural Resources, Ahwaz, Iran

Then, for digestion by enzyme and determination of RFLP, 10 μL of PCR product (776 bp) was added to 1 μL of MspI enzyme and 2 μL buffer 10X and final volume was 30 μL with dilution water and digested in ban marry bath for night. Digested samples were loaded by 2.5% agarose gel with etidium bromide for 90 sec with voltage 70, then genotypes were scored.

RESULTS AND DISCUSSION

DNA extraction and quality determination with electrophoresis and spectrophotometer was done. Obtained result was acceptable (Fig. 1).

PCR amplification was carried out using primers written in Table 1. The 776 bp was amplified by primers in total samples under condition that explained in previous section (Fig. 2).

With electroforesing of digested samples, 3 types of genotype were diagnosed. In AA genotype 125, 237, 414 bp bands were registered. In AB genotype 237 and 539 bp bands and in BB genotype 776 bp band were determined.

In total, 39 samples have AA genotype, 59 samples have AB genotype and 2 samples shown BB genotype (Fig. 3).

The band 539 has been existed by mutation. This mutation was reported by Stephen *et al*. (2001) that researched on 28 populations of native chicken chiness. Genotype and gene frequency in population is shown in Table 2. Calculations were done with Popgene programe.

The observed heterozigosity was 0.59 in this study, in population and the mean effective number of alleles

Table 1: Primer sequence using in PCR

Primer	Sequence
Forward	5'-ATCCCCAGGCAAACA ICC IC-3'
Reward	5'-CC ICGACA ICCAGC ICAC A I-3'

Table 2: Obtained genotype and gene frequency of MspI Enzyme on 776 bp fragment

Genotypes	No.	Frequency
AA	39	0.390
AB	59	0.590
BB	2	0.020
Allele		
B	137	0.685
A	63	0.315

Table 3: Calculating Hardy -Weinberg equilibrium in population

Genotypes	Observed individuals (O)	Expected individuals (E)	$O-E^2/E$	χ^2
AA	39	46.92	1.34	13.47
AB	59	43.16	5.81	-
BB	2	9.92	6.32	-

Fig. 1: DNA extracted samples

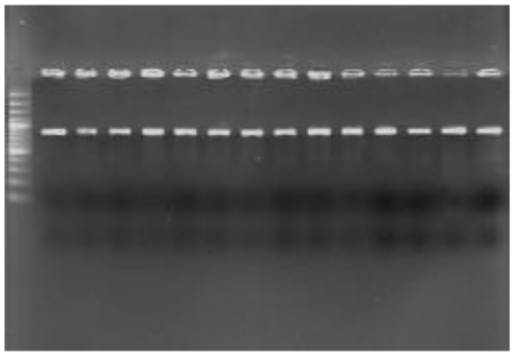

Fig. 2: Electrophoresed PCR product on agarose gel: marker 100 bp

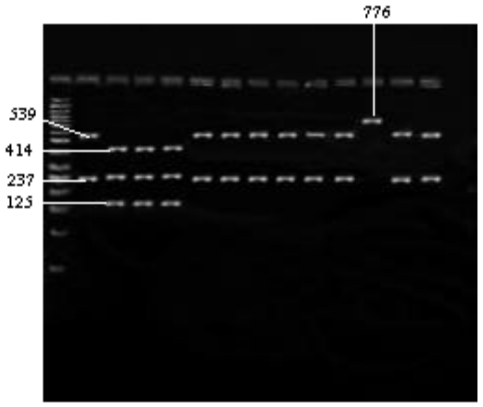

Fig. 3: Electroforesing digested product on agarose gel: marker 50 bp

was 1.7592. Hardy-Weinberg equilibrium was investigated and result was shown that this population has not Hardy-Weinberg equilibrium (Table 3).

CONCLUSION

Results show that used selection criterias in native population breeding, has been related with performance of growth hormone gene.

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