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Short Communication

POLYMORPHISM OF INSULIN-LIKE GROWTH FACTOR-1 GENE AND ITS ASSOCIATION WITH GROWTH RATE IN DESI CHICKEN OF PAKISTAN

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ABSTRACT

The association of insulin-like growth factor-1 (IGF-1) polymorphism with growth trait was investigated in native Desi chicken of Pakistan. The present study was conducted at Animal Genetics Laboratory of Department of Livestock Production, UVAS Lahore. Fifty Desi chicken were procured from a conventional production farm situated in the vicinity of Ravi Campus for phenotypic and genotypic analysis. Genomic DNA was extracted from growing feather pulp using standard Phenol Chloroform Isoamylalcohol (PCI) method and the polymorphism of IGF-I gene was detected by PCR-RFLP-PstI. Both allelic and genotypic frequencies were calculated with the help of Pop gene 1.32 software. Statistical analysis of data was done using LSD (least significant difference) method as applied in the General Linear Model (GLM) procedure (SAS 9.1). Genotyping of birds revealed two alleles A (364 and 257 bp), B (621 bp) and three genotypes AA (6), AB (32), BB (12). AB genotype have the highest frequency of 64% while for BB and AA the frequencies are 24% and 12% among selected Desi Chicken. Analyzing different genotypes reveals that AA individuals had highest six month body weight (g) (1156.33±31.17) followed by (1112.16±21.04) and (904.08±31.08) of AB and BB genotypes respectively. Based on the results of this study, the application of IGF-1 locus in Marker Assisted Selection programs can be advised.

Key words: IGF-1, Polymorphism, Body weight, Desi chicken, Pakistan.

INTRODUCTION

Indigenous or native poultry plays very important role in the strengthening of economy of backyard peoples, it is source of food and employment for small poultry keepers without investing a penny on the management, disease control and nourishment (Ekue *et al.* 2002). Native poultry survives well in their local environment and can be reared on kitchen waste and may be as free range on open lands. Although commercial poultry has taken its place but native poultry is still playing very crucial role in the economy of third world countries like Pakistan and extensive work is required to improve the economic traits of native chicken through modern techniques that helps in selection (Iqbal *et al.* 2012).

Desi chicken are very commonly found in almost every village of Pakistan, although these chickens are known as poor egg producer with smaller egg size and poor feed converter but are still very common and abundant because they are reared as scavenging birds (Sahota and Bhatti, 2002). As these chicken are reared by poor people under very miserable conditions so their productive and reproductive potential is not an actual representation of their genetic potential. So in order to make this breed commercially viable both genetic and nutritional interventions are needed. Detection of molecular makers in these low producing breeds and then incorporation of that information in breeding plans may be very helpful for getting high producing better chicken (Zhou *et al.* 2005).

Insulin-like growth factor-1 is a mitogenic polypeptide hormone having structural, functional similarity to insulin with many metabolic and anabolic properties. IGF-1 plays major role in cellular growth by intervening many of the actions of growth hormone and can affect a wide range of biological processes, ranging from body growth and differentiation to reproduction in poultry (Lei *et al.* 2005; Zhou *et al.* 2005; Tang *et al.* 2010). The chicken IGF-1 gene has been mapped on chromosome 1 (165.95 centi-morgan) and the QTLs affecting body weight, abdominal fat has been found within the same region having confidence interval within 100-182 centi-morgan (Ikeobi *et al.* 2002; Sewalem *et al.* 2002). This is why it has been used as candidate gene for growth traits in many studies and in this study too.

MATERIALS AND METHODS

A total of 50 Desi chicken were used in this study. Phenotypic values of body weights at 1st, 2nd, 4th and 6th months of age were recorded. Growing feathers were collected from all experimental birds for genomic DNA extraction.

DNA extraction and quantification: Genomic DNA (gDNA) was extracted from growing feather pulp using

standard Phenol Chloroform Isoamylalcohol (PCI) method (Sambrook *et al*. 1989). The extracted DNA was evaluated through gel electrophoresis and brought to same concentration 50 ng/µL.

PCR-RFLP-Pst1: Extracted gDNA of each individual was suspended in low TE at final concentrations of 50ng/µL. The Primer sequences used for the amplification of IGF-1 were same as used by Nagaraja *et al.* (2000).

PF 5′-GAC TAT ACA GAA AGA ACC CAC-3′
PR 5′-TAT CAC TCA AGT GGC TCA AGT-3’

The amplification of IGF-1 was carried out in total volume of 25 µL, containing 2 µL (50ng/µL) of gDNA, 1 µL of each 10 pM oligonucleotide primers, 2 µL of 2 mM $MgCl_2$, 2.5 µL of 25mM deoxynucleotide triphosphate mixture, 2.5 µL 2 mM of *Taq* buffer, 0.2 µL of 5U/µL *Taq* DNA polymerase and 13.8 µL of distilled water. Amplification reaction conditions includes, initial denaturation of gDNA at 94 °C for 5 min and then 35 cycles of 94 °C for 45 sec, 57 °C for 45 sec, and 72 °C for 45 sec, with a final extension step for 10 min at 72 °C. The amplified amplicons of 621 bp were subsequently digested by PstI and digests were separated using 2.5% agrose gel in 1x TAE at a constant supply of current. The gels were stained with ethidium bromide and the fragments were visualized with the help of UV transilluminator. UV illumination revealed two alleles A, B and three genotypes. Allele B was designated to fragment of 621 bp and allele A to the fragments of 364 and 257 bp.

Statistical analysis: Both allelic and genotypic frequencies were calculated with the help of Pop gene 1.32 software. The effect of different genotypes on body weight was calculated assuming following mathematical model.

$Y_{ij} = \mu + g_i + \epsilon_{ij}$

Where Y_{ij} is the phenotypic value of body weight, μ is the population mean for body weight, g_i is the fixed effect of i^{th} genotype (i = 1-3) and ϵ_{ij} counts the residual effects associated with j^{th} observation of i^{th} genotype.

Least significant difference (LSD) test was applied in General Linear Model (GLM) procedure of SAS (9.1) for evaluating statistical differences among mean weights of different genotypes.

RESULTS

The PCR-RFLP analysis of gDNA of Desi chicken showed three types of genotypes, named AA (364+257), AB (621+364+257) and BB (621) at SNP (IGF-1-Pst1) within 5’ flanking section of IGF-1 gene as shown in figure 1. The genotypic and allelic frequencies of IGF-1 in Desi chicken are given in the Table 1. The highest observed genotypic frequency was of heterozygous AB (64 %) followed by 24 % and 12 % of BB and AA genotypes. It is also evident from the Table 1 that the frequency of allele B is higher than allele A in the population of Desi chicken.

Association of observed genotypes with body weights at different ages is showed in Table 2. In Desi chicken the body weight of AA genotype showed significantly higher values than both AB and BB genotypes at the age of 1st, 2nd, 4th months of age while there was non-significant variation at the age of 6th month between AA and AB.

Body weight (g) (328.12±5.58) of AB (heterozygous) birds at the age of 1st month were non-significantly varied from 311.41±6.07 of BB but later on AB individuals showed significantly higher body weights than BB (non-mutated) individuals.

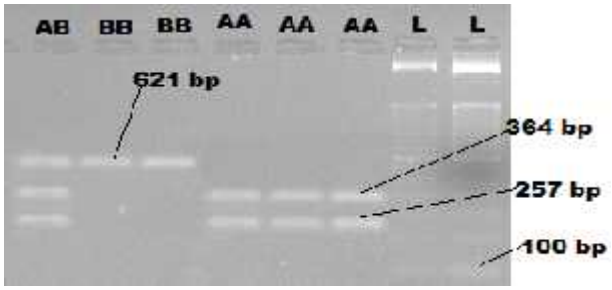

Figure. 1. PCR-RFLP Pst1 digests on 2.5 % agarose gel with 100 bp ladder

Table. 1. Genotypic and allelic frequencies of IGF-1 gene in Desi chicken

Breed	Genotypic frequencies (%)			Allele frequencies (%)	
	AA	AB	BB	A	B
Desi	12 (6)*	64 (32)	24 (12)	44	56

*figures in the brackets are the number of individuals.

Table. 2. Effect of different genotypes on body weight of 4 different ages

Genotypes (number)	AA (6)	AB (32)	BB (12)
1st month BW (g)	356.33±12.28^a	328.12±5.58^b	311.41±6.07^b
2nd month BW (g)	557.5±9.1^a	516.62±7.75^b	470.83±11.77^c
4th month BW (g)	860.83±14.85^a	762.03±11.03^b	656.16±9.5^c
6th month BW (g)	1156.33±31.17^a	1112.16±21.04^a	904.08±31.08^b

*Means with same letter on superscript within the same row are not significantly different (P<0.05).

Growing trend of Desi chicken with different genotypes is given as Fig: 1. Graphical representation revealed that the body weight of AA is higher than AB and BB at 1st, 2nd, 4th, 6th month of age in Desi chicken of Pakistan.

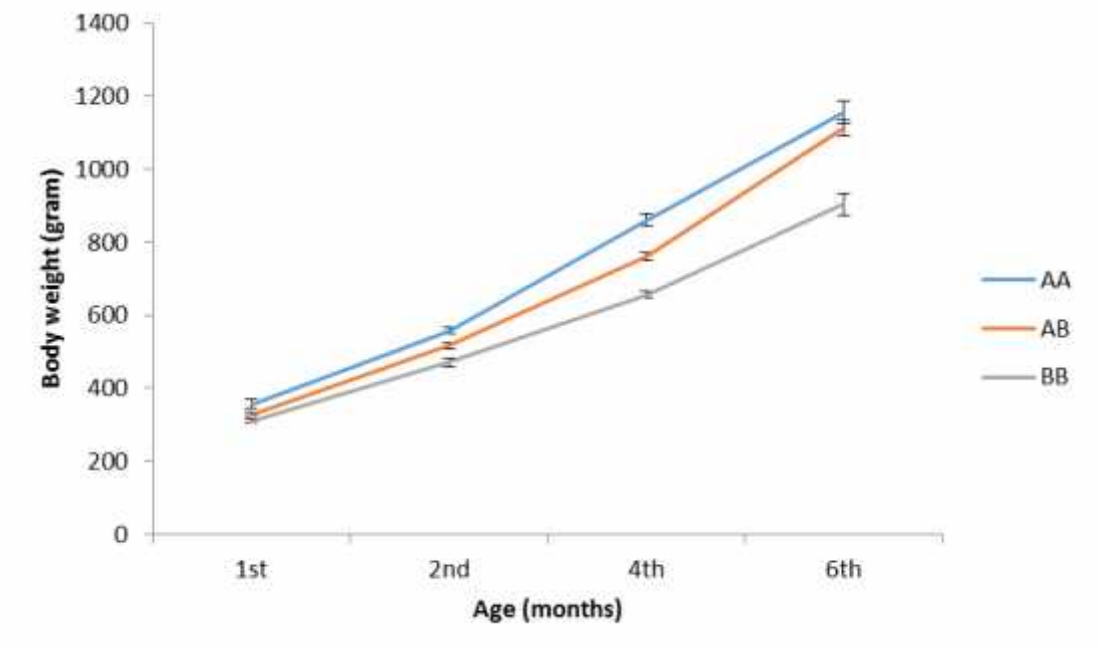

Figure: 1. Growth curve analysis of Birds with different genotypes

DISCUSSION

The candidate gene approach is the most recent and accurate method to find polymorphisms of the genes that are economically more important i.e. genes that are related to growth and egg production in chicken (Zhou *et al.* 2005). IGF-1 gene in chicken is involved in a very complex, crucial regulatory network just like insulin for cell maintenance, maturation, cell reproduction and ultimately helps in growth of tissues (McMurtry *et al.* 1997; Duclos *et al.* 1999; Kadlec *et al.* 2011).

Thus IGF-1 is known as a very positive modulator for body growth and muscle development in many species. The chicken IGF-1 gene has been shown to be at the short arm of chromosome 1 near the centromere and to be conserved in several vertebrate species (Klein *et al.* 1996). Also, the RFLP analysis of this gene has shown a single PstI polymorphism in the 5` flanking region and its association with growth like traits has been studied i.e. reduction in abdominal fat content, body muscles, body measurements and body weight at various ages (Tang *et al.* 2010).

Digestion of amplified 621 bp product of IGF-1 reveals the existence of polymorphic fragments with the length of 257bp, 364bp and 621bp, which is in consistent with the previous reports by Nagaraja *et al.* (2000), Wang *et al.* (2004) and Abbasi and Kazemi, (2011).

Analysis revealed that the Desi chicken carries high frequency of allele B (0.56) than allele A (0.44). Lower frequency of allele A is in line with the lower frequency of allele A (0.43) in Korean Native Ogol chicken as observed by Seo *et al.* (2001). As Desi birds have slower growth rate so these results are in line with the study conducted by Sato *et al.* (2012) who concluded that the high breast muscle producing birds have higher frequency of allele A (1.0) as compared to low breast muscle producing chickens having (0.29) frequency of allele (A). Lower frequency of allele A in Desi (known as low producer) as compared to allele B is also supported by another study conducted on 12 Asian native chicken populations and two high yielding broiler breeds where it ranged from 0 to 0.52 in native populations and 0.86 and 1.00 in Cobb and Chunky broiler breeds (Moe *et al.* 2009).

Association of genotypes with body weight at different ages indicated that individuals carrying IGF-1-PstI had the higher body weight as compared to wild types (without polymorphism) (P> 0.05). In Wanzhai Yellow and Ningdu Yellow chicken breeds IGF-1-PstI showed higher body weights as studied by Wang *et al.* (2004). Nagaraja *et al.* (2000) and Seo *et al.* (2001) have also studied the association of IGF-1 SNP with body weight at different ages and they also concluded the influential trend of IGF-1 SNP on body weight as similar as observed in Desi chicken.

In the context of aforementioned discussion it can be concluded that the SNP in IGF-1 have role in controlling overall growth rate in chicken and can be a genetic marker for selection in Desi chicken breeding systems aimed to develop meat type lines.

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