Genotypic and Allelic Frequencies of *IGF1* and *IGF2* Genes in Broilers Analysed by Using PCR-RFLP Techniques

Research Article   **T. Shah[1], S. Deshpande[2*], U. Dasgupta[2], K.M. Singh[3] and C.G. Joshi[1]**

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ABSTRACT

A polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) test was performed to investigate the allele frequencies of the insulin-like growth factor (*IGF1* and *IGF2* genes) in 90 broiler chickens. A 793 bp fragment of *IGF1* gene and 1146 bp fragment of *IGF2* gene were amplified and digested with *Hinf*I and *Hsp92*II restriction enzymes, respectively. Two types of alleles of A and B and three types of genotypes of AA, AB and BB were observed in both genes. The results indicated predominant B-allele (0.85) was higher for *IGF1* but A-allele (0.5611) IGF2 in the broiler population.

KEY WORDS genotype, *IFG1*, *IGF2*, PCR-RFLP.

INTRODUCTION

Insulin-like growth factor-1 (*IGF-1*) is important for regulating animal body growth, development and metabolism. Growth is a complex process that involves the regulated coordination of a wide diversity of neuroendocrine pathways. Insulin-like growth factors (*IGF*) and their receptors are essential players in this biological process by modulating intermediary metabolism and cell proliferation. Chicken insulin-like growth factors 1 and 2 (*IGF1* and *IGF2*) are polypeptidic hormones that exert their function by binding to specific type 1 receptors (Zhou *et al.* 1995). The metabolic effects of avian *IGF*s include an increased amino acid and glucose uptake and the upregulation of DNA and protein synthesis (McMurtry, 1998). Plasma levels of *IGF1* and *IGF2* decrease with fasting and increase with age (Beccavin *et al.* 2001).

Moreover, there is ample evidence suggesting that IGF might influence growth rate, body composition and lipid metabolism in poultry (McMurtry, 1998; Tomas *et al.* 1998; Beccavin *et al.* 2001).

The molecular characterization of the chicken *IGF1* and *IGF2* has provided valuable clues for understanding how they are regulated and expressed. The chicken *IGF1* gene maps to chromosome 1 and encompasses 50 kb (Kajimoto and Rotwein, 1991; Klein *et al.* 1996). Multiple alternative promoters and two different variants generated by alternative splicing have been reported (Kajimoto and Rotwein, 1991). The profile of *IGF1* mRNA expression is remarkably ubiquitous and includes liver, muscle, kidney, testes, heart, ovary, brain, intestine and other tissues (Tanaka *et al.* 1996; McMurtry *et al.* 1998). The *IGF2* gene contains three exons and maps to chromosome 5 (Darling and Brickell, 1996; Yokomine *et al.* 2001). In mammals, the *IGF2* gene is paternally imprinted (DeChiara *et al.* 1991; Killian *et al.* 2001). In contrast, the chicken *IGF2* gene displays a biallelic pattern of expression (O′Neill *et al.* 2000; Nolan *et al.* 2001; Yokomine *et al.* 2001). Association studies between

mutations at the *IGF1* and *IGF2* genes and productive traits are very scarce in chicken. Nagaraja *et al.* (2000) described one *Pst*I RFLP in the 5′ end of the *IGF1* gene that was associated to egg and egg shell weights in an unselected White Leghorn chicken population. Moreover, Yan *et al.* (2002) reported an association between phenotypic variation at several growth and carcass traits with one polymorphism in exon 2 of the chicken *IGF2* gene. The objective of the present study was to identify polymorphisms of *IGF1* and *IGF2* genes in broiler birds produced by Marshall Breeders Pvt. Limited.

MATERIALS AND METHODS

The present study was carried out on 90 selected broilers maintained at FCR trial farm of the company. Blood samples (approximately 0.5-1 mL) were collected in vacutainer tubes containing EDTA. The vials were kept immediately in ice-boxes containing ice and gel cool packs. Samples were kept in deep freeze at -20 °C till the isolation of DNA.

DNA Extraction
The phenol-chloroform extraction protocol was followed as per John *et al.* (1991) with some modifications (there is no need of using RBC lysis buffer and separation of buffy coat in the modified method). Briefly, a volume of 100 μL of blood was transferred to a sterile centrifuge tube containing 400 μL extraction buffer (10 m*M* tris HCL, 10 m*M* EDTA and 1% SDS, pH 8.0), 10 μL proteinase K (10 mg/mL) and 5 μL RNase (10 mg/mL) and thoroughly mixed by inversion. The solution was then incubated at 55 °C for 2 h with frequent mixing every 10 min. Subsequently, an equal volume of phenol-chloroform iso-amyl alcohol (25:24:1) mixture was added to the above solution and mixed thoroughly by inversion. Thereafter, the mixture was centrifuged at 10000 rpm for 10 min at 8 °C and the supernatant was transferred to a fresh tube. Then an equal volume of chloroform was added and mixed thoroughly by inversion. The mixture was centrifuged at 10000 rpm for 10 min at 4 °C and the supernatant was again transferred to a fresh microcentrifuge tube. Two volumes of chilled iso-propanol and 1/10 vol of 3 M sodium acetate (pH 7.2) were added to the above supernatant and mixed by inversion. Subsequently, the tube was kept at -20 °C for overnight. Thereafter, the contents were centrifuged at 12000 rpm for 15 min. The supernatant obtained was discarded leaving the pellet in the tube. The pellet was washed twice with 70% alcohol and air dried. The extracted DNA was dissolved in 100 μL of TE buffer and stored at -20 °C. The quality of extracted DNA was checked by performing horizontal electrophoresis on 0.8% (w/v) agarose gel, visualized under UV transilluminator and documented through gel documentation sy-

stem. The purity of the extracted DNA was checked by a Nanodrop spectrophotometer at 260 and 280 nm. The DNA samples exhibiting an OD_{260} / OD_{280} ratio between 1.7-1.9 only were selected for further analysis.

Polymerase chain reaction and RFLP
As reported by Amills *et al.* (2003), the 793 bp DNA fragment was amplified by polymerase chain reaction (PCR). The genotype and allele frequencies were estimated by using SAS software. Similarly for IGF2 locus, an 1146 bp product was also amplified by using primers GALIGF2 5′-CCA GTG GGA CGA AAT AAC AGG AGG A-3′ and GALIGF2-5′-TTC CTG GGG GCC GGT CGC TTC A-3′ (Amills *et al.* 2003). The genotype and allele frequencies were estimated as described above.

RESULTS AND DISCUSSION

The sequences of the *IGF1* and *IGF2* genes have been amplified successfully by using genomic DNA samples. The restriction digestion analysis of 793 PCR product of *IGF1* indicates the presence of two restriction patterns. In the first pattern fragments 620 / 173 bp was observed while in the second pattern three fragments 378 / 242 / 173 bp was observed (Figure 1) and first pattern was assigned as genotype BB; second pattern as genotype AA.
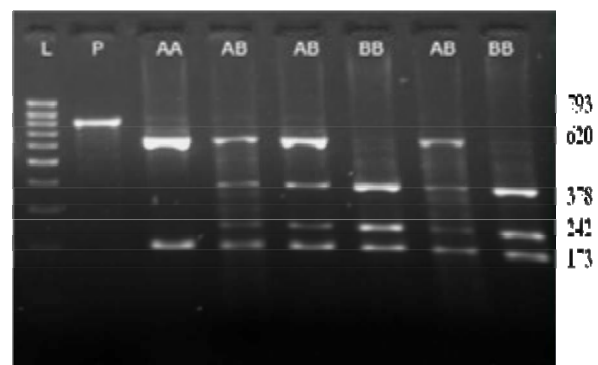

Figure 1 Agarose gel electrophoresis (2%) showing genotyping profiles of *IGF1* gene detected by PCR-RFLP method. L: 100 bp DNA Ladder

Digestion of the PCR product of *IFG2* also yielded two restriction patterns named as A (246/39/138/30/693 bp) and B (246/39/138/30/478/215 bp) (Figure 1). The estimated genotypes, genotype frequencies and allelic frequencies of *IGF1* and *IGF2* are presented in Table 1.

The genotype frequencies observed in the samples were 0.066, 0.166 and 0.766 and allelic frequencies were 0.15 and 0.85 respectively for *IGF1* (Table 1). Also the genotype frequencies were observed in the samples is 0.3889, 0.3444 and 0.2667 and allelic frequencies were 0.5611 and 0.4389 respectively for *IGF2* (Table 1).
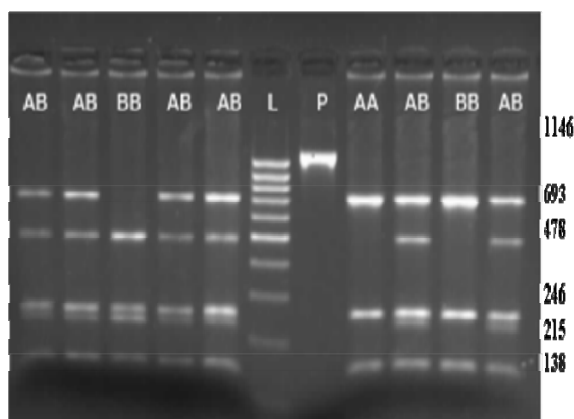
Figure 2 Agarose gel electrophoresis (2%) showing genotyping profiles of *IGF2* gene detected by PCR-RFLP method. L: 100 bp DNA Ladder

Similar observation has been also reported by Amills *et al*. (2003) in Black Penedesenca chicken strain. The highly significant Chi square value in the sample showed that the population was not in Hard Weinberg equilibrium for both the genes (Table 1).

Table 1 Obtained genotypes, and Alleles frequency of *Hinf*I restriction enzyme on 793 bp fragments and *Hin1*II restriction enzyme on 1146 bp

Genotype	Frequency	
	IGF1	IGF2
AA	0.0667	0.3889
AB	0.1667	0.3444
BB	0.7667	0.2667
Alleles		
A	0.1500	0.5611
B	0.8500	0.4389
Chi-square value	10.7997	8.1359
Heterozygocity	0.1667	0.3444
Allelic diversity	0.2550	0.4925

Amills *et al*. (2003) suggested that the sequence alignment of the chicken IGF sequences revealed the existence of three SNP. One SNP (IGF1-SNP1) was located in the 5′UTR of the IGF1 gene and consisted of one A→C substitution. This mutation was associated with a *Hinf*I RFLP. Moreover, two SNP were found in the IGF2 gene. One of them was a neutral substitution C→T at exon 3 (IGF2-SNP2) and could be detected with the *Hsp92*II restriction enzyme. The second SNP (IGF2-SNP3) was a G→A substitution at intron 2.

Three SNP (IGF1-SNP1, IGF2-SNP2 and IGF2-SNP3) described by Nagaraja *et al*. (2000) and observed that the genotype frequencies between strains were similar to our observation. The present results only described the genotypes and allelic frequencies in poultry. In this way, several reports indicate that *IGF1* is more intimately linked to post-hatch development than *IGF2*. For instance, Tomas *et al*. (1998) used human recombinant *IGF1* and *IGF2* into three

different strains of chickens (fat, lean, and control) and demonstrated that *IGF1*, but not *IGF2*, significantly increased growth rate. Similarly, it has been observed that hepatic IGF1 mRNA levels are higher (Beccavin *et al*. 2001) and earlier expressed (Wu *et al*. 2011) in the high-growth strain as compared to low-growth strain. Gouda and Essawy (2010) analyzed the polymorphism of IGF-I gene among Egypt chicken breeds and indicated their effect on the growth traits of chicken was significant. IGFBP2 and STAT5b act as modulators for the biological action of IGF gene in various signalling pathway, so their expression level as well as SNPs play important role in the action of IGF protein. Studies of the IGFBP-2 biological function showed that reduced growth of mice selected for low body weight was associated with increased hepatic IGFBP-2 mRNA expression and elevated serum IGFBP-2 levels (Hoeflich *et al*. 1999). In chickens, Leng *et al*. (2009) detected one SNP (C1996A) of the IGFBP-2 gene was associated with abdominal fat weight and percentage of abdominal fat. Li *et al*. (2006) detected a C / T SNP of the IGFBP-2 gene which had effect on BW, metatarsus length, shank length, femur length, shank weight, femur weight, metatarsus claw weight and abdominal fat weight of chicken. Dietary supplement like methionine feed to protein starved chickens has alleviated body weight loss and not improved by glycine supplements, and the effect was independent of the change in *IGF1* plasma concentration (Nagao *et al*. 2011). Literature suggested that *IGF1* is mainly involved in fat deposition and lipid metabolism (Huybrechts *et al*. 1992; Tixier-Boichard *et al*. 1992; Spencer *et al*. 1995). The search for mutations not only in the *IGF1* and *IGF2* coding and regulatory sequences but also in their modulators (IGFBP2 and STAT5b) also plays an essential role for elucidating the molecular basis of these associations. Considering the scientific report, m RNA expression profiling of IGF gene could be useful for association with body weight and feed conservation.

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