Indian J. Anim. Res., 50 (1) 2016: 23-26
Print ISSN:0367-6722 / Online ISSN:0976-0555

AGRICULTURAL RESEARCH COMMUNICATION CENTRE
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Molecular characterization of promoter of IGF-1 gene in chicken broiler line and its growth performance

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Received: 28-06-2014 Accepted: 12-03-2015 DOI:10.18805/ijar.8417

ABSTRACT

A study was carried out to characterize the nucleotide variability in the promoter of the IGF-1 gene in broiler line of chicken. A PCR product of 375bp was amplified and nucleotide variability was studied using PCR-SSCP technique in chicken control broiler line. Selected sample PCR products were also sequenced to confirm the variability in promoter sequence. Present study revealed that the IGF-1 promoter was monomorphic having similar SSCP pattern in all individuals. Growth data was also analyzed to study the growth performance of the chicken broiler line at different age. Growth performance of male and female differed significantly at six week of age.

Key words: Chicken, Growth performance, IGF-1 promoter, Nucleotide variability.

INTRODUCTION

In the past decade, poultry industry has emerged as a big hope, which has potential of not only to meet the protein requirements to millions but can also provide employments and thereby livelihood security (DAHD, 2011). In a realistic estimate, we need to enhance existing poultry production at least by three folds to meet the minimum nutritional standard set by Indian Council of Medical Research (ICMR). In India, the per capita consumption of egg and meat are only 61 and 1.6 kg respectively, against the minimum recommendations of 180 eggs and 11 kg meat per year per person by Indian Council of the Medical Research (Annual Report, DAHD, 2014-15).

Till date, most of the genetic progresses for economic traits like body weight and egg production in poultry have been made by selection on phenotype without the knowledge of gene/genes controlling those traits. This approach of quantitative genetics has lead to tremendous progress in poultry industry across the globe. But, conventional breeding methods have certain limitations like slow progress, requirement of selectable phenotypic variation, large population size etc.

In broilers, selection of birds for higher body weight in shorter period has been the emphasis in conventional breeding programs. Due to limited molecular investigations in this line, the molecular mechanism behind the muscular growth is a mystery even now. Quest for unraveling the genetic mechanism controlling the growth of the muscle has resulted in the development of several protein factors regulating the muscles growth. IGF-1 is one of the important protein factors regulating the muscular growth in chicken (Duclos *et al*., 1993; McMurtry, 1998; Arnold *et al*., 2001).

The insulin-like growth factor 1 (IGF-1) is a polypeptide hormone structurally related to insulin with multifunctional metabolic activities that exerts its function by binding to specific type I trans-membrane receptors (Zhou *et al*., 1995). The IGF-I stimulates glucose uptake, amino acid uptake, protein synthesis etc. and inhibits protein degradation by satellite cell derived myotubes (Duclos *et al*., 1993, McMurtry, 1998). The IGF-1 stimulates the proliferation, differentiation and metabolism of myogenic cells (Florini *et al*., 1996). The IGF-1 gene plays important roles in growth of multiple tissues, including muscle cells (Zapf and Froesch, 1999).

Although, in recent past, advancements in molecular genetics tools have facilitated understanding the functional mechanism of the IGF-1 gene, little is known about the nucleotide variation in the promoter region of this gene and its effect on regulation of this gene. Therefore, this study was carried out to study the nucleotide variation in the promoter region of the IGF-1 gene and its association with the body weight.

MATERIALS AND METHODS

Experimental birds: A total of 206 birds, of fast growing chicken control broiler line (CB), raised at ICAR-Directorate of Poultry Research (ICAR-DPR) farm, Hyderabad were included in the present experiment. CB has been bred randomly without practicing selection and is extensively used for estimation of phenotypic and genetic effects in the selected line due to application of selection for a specific trait. All the birds were reared on deep litter system in the same shed under intensive management of farming, providing same management regime with *ad-lib* feeding and watering.

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All the birds were hatched at the same time and housed all along in the same shed. Cooling facilities were provided during summer season through water sprinkling on the roof and proper lighting were arranged in the shed so that birds get congenial environment for performing in optimum potential.

Collection of blood samples: 0.5-1 ml of blood was collected from the wing vein of each bird in a sterile polypropylene 2ml centrifuge tubes containing 2.7% EDTA (60-70µl/1 ml of blood) as an anti-coagulant . Vials were kept immediately on ice and transported to the laboratory immediately. Samples were kept in deep freeze at –20°C till the isolation of DNA.

Extraction, quality and purity of genomic DNA: Genomic DNA was isolated from the blood samples by Phenol: chloroform extraction method as described by Sambrook and Russell (2001). Horizontal submarine 0.8% agarose (w/v) gel electrophoresis was performed to check the quality of genomic DNA. On completion of electrophoresis, the gel was visualized under UV transilluminator and documented by gel documentation system. DNA samples showing intact band and devoid of smearing were used for further analysis. The purity of genomic DNA was checked by using UV-Spectrophotometry. Genomic DNA samples lying in the ranges of OD ratio (260:280) between 1.7 to 1.9 were considered good and were used for further study (PCR amplification).

Designing of primers for amplification of IGF-1 promoter: A forward (5'-3') AGATGTACACAGGAATATAAAGG and reverse (5'-3') CTA CATTACACAGACACTGTAG primers, used for amplification of IGF-1 gene partial promoter was designed using DNAstar laser gene software.

PCR amplification of IGF-1 promoter: A PCR reaction of 25µl was set up using 10 x PCR assay buffer, dNTPs mix (2.5 mM), primers (forward and reverse) 40ng each, $MgCl_2$ (25 mM), Taq DNA polymerase 0.3 units (Fermentas USA) and genomic DNA (80-100 ng) of individual bird. IGF-1 gene was amplified using initial denaturation at 95^{0}C for 5 min, followed by 35 cycles of denaturation at 95^{0}C for 30 sec, annealing at 58.4 ^{0}C for 30 sec and extension at 72 ^{0}C for 1 min. The final extension used in this amplification was at 72 ^{0}C for 10 min.

Nucleotide variability study: The SSCP was carried out on 12% native PAGE (50:1, acrylamide and bis-acrylamide) with 5% glycerol. A volume of 3 µl PCR product mixed with 15 µl formamide dye [95% formamide, 0.025% xylene cyanol, 0.025% bromophenol blue, 0.5M ethylene diamine tetra-acetic acid (EDTA)] was denatured at 95^{0}C for 5 minutes followed by snap cooling on ice for 15 min. Then, the product was loaded in the gel and electrophoresis was performed at 4^{0}C for 12 hrs at 200 V. After electrophoresis was over, the gel was stained with silver nitrate to visualize banding patterns of the fragments (Bhattacharya *et al*, 2013).

PCR products amplified in this study using HotStar HiFidelity DNA Polymerase (MBI Farmentas) were sequenced using fragment-specific primers from both ends by the automated dye–terminator cycle sequencing method in ABI PRIZM 377 DNA sequencer (Perkin-Elmer).

Growth performance: Body weights at hatch (do), second week (wk2), fourth week (wk4) and sixth weeks (wk6) were recorded. Data was analyzed by least squares linear model using SAS 9.3 software, where sex was considered as fixed factor and traits (body weights at different age) were taken as dependable factors.

Thus the model used for this analysis was: $Y_i=\mu+ S_i+e_i$

Where, µ is mean of the parameter, S_i= ithsex (Male or Female) and $e_{i=}$random error

RESULTS AND DISCUSSIONS

Polymorphism of IGF-1 partial promoter : A PCR product of 375bp of IGF-1 promoter was amplified in control broiler line. PCR-SSCP results revealed monomorphic pattern of IGF-1 promoter in control broiler line (Fig1). Similar observation was reported in other region of the IGF-1 promoter sequence (Paswan et al., 2013). This observation was in contrast to one study which reported polymorphism in promoter as well as 5' UTR of IGF-1 gene (Hossein and Mohsen, 2011). Bhattachrya *et al.* (2013) reported 16 haplotypes in the coding regions of the IGF-1 in chicken.

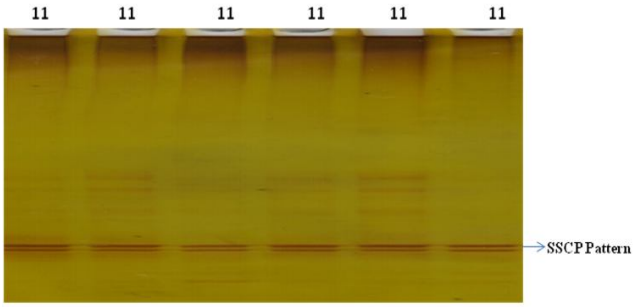

Fig. 1: SSCP Patterns of IGF1 in CB

Nucleotide variability of IGF-1 promoter : PCR products amplified in this study were sequenced using fragment-specific primers from both ends by the automated dye–terminator cycle sequencing method. Sequenced nucleotides were compared with the original sequence from which primer was designed, present study revealed transition mutation at 27 A>G. It was observed that this mutation was common to all the individuals of the population that may be reason of whole population being monomorphic for IGF-1 promoter fragment (Fig2). Paswan *et al.* (2013) reported transition mutation in other region of the IGF1 promoter in chicken. This is in contrast to earlier findings which reported polymorphisms in several regions of promoter as well as coding regions of IGF-1 gene (Amills *et al.* 2003; Zhou *et al.* 2005; Li *et al.*, 2010).

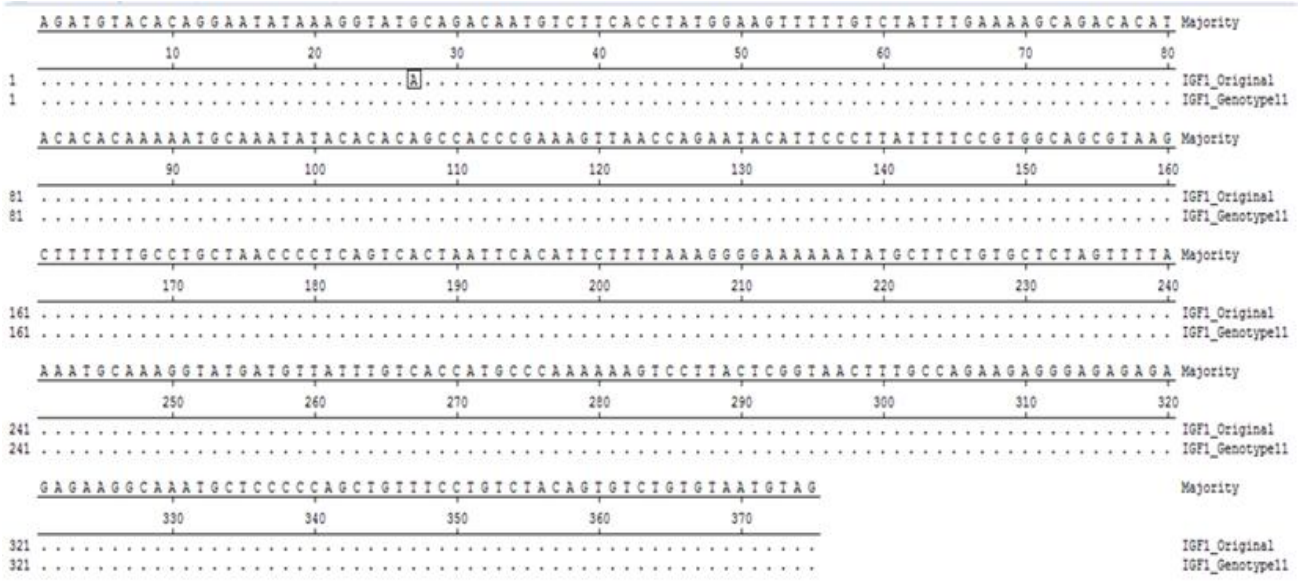

Fig2: Alignment of IGF1 genotype11 with original sequence

Growth performance: The body weight of male at do, wk2, wk4, wk5 and wk6 were 43.32±2.40g, 118.08±2.17g, 377±7.37g, 583.64±10.41g and 875.18±14.52, respectively. Weight of females at corresponding age was 39.37±2.57g, 117.61±2.32g, 372.03±7.97g, 572.52±11.31g and 816.28±15.88g. Body weight of male and female differed significantly at sixth week of age (wk6) Table1. Eid *et al*. (2010) reported that there was no difference of body weight between male and female at 5th and 6th week of age but differed significantly at 7th week

TABLE 1: Least Squares means of Body Weight at different Age in Control Broiler

Parameters	Male	Female	p-value
Bwt (d0) ±SE	43.32±2.40[a]	39.37±2.57[a]	0.27
Bwt (wk2) ±SE	118.08±2.17[a]	117.61±2.32[a]	0.88
Bwt (wk4) ±SE	377±7.37[a]	372.03±7.97[a]	0.64
Bwt (wk5) ±SE	583.64±10.41[a]	572.52±11.31[a]	0.47
Bwt (wk6) ±SE	875.18±14.52[a]	816.28±15.88[b]	0.007

Bwt (body weight), d0 (hatch day), wk2 (second week), wk4 (fourth week), wk5 (fifth week) and wk (sixth week). SE (Std Error). Superscript with same alphabet indicates non-significant difference along row and different superscript indicates significant difference along row.

CONCLUSION

IGF-1 partial promoter was found to be monomorphic. The growth performance of male and female differed significantly at sixth week of control broiler line of chicken. PCR-SSCP result was confirmed by sequencing of the PCR product which gave similar result as that of SSCP.

ACKNOWLEDGMENT

We acknowledge the ICAR-Directorate of Poultry Research Hyderabad, for providing necessary facilities to carry out this research work.

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