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SHORT COMMUNICATION

Characterization of Exon4 of FSTN Gene and its Association with Growth Traits in PD-1 Broiler Chicken

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ABSTRACT

Follistatin play vital role in biological processes which includes cell proliferation, differentiation, and skeletal muscle growth. The present study was carried out to study polymorphism of exon4 of follistatin gene and its association with body weight in PD-1, a broiler line of chicken. A product of 225 bp of exon-4 was amplified and structural variability was studied using polymerase chain reaction, single strand confirmation polymorphism and sequencing method. This study revealed that the FSTN gene was monomoprphic at exon4. Growth data was also analyzed, the growth performance of male and female differed significantly at six week of age.

Keywords: Chicken, growth performance, FSTN Exon4, nucleotide variability

Follistatin (FSTN) is one of the members of TGF-β super family regulating muscle growth in chicken through cell proliferation, differentiation, (Ampthor *et al.,* 1996). Follistatin is distributed in embryonic and adult tissues and is not confined to reproductive tissues. It regulates the activity of myostatin and control the division of myoblasts in the myotome (Currie and Ingham *et al*., 1998). It is also present in circulatory system and binds to activin via their β subunits (Patel *et al*., 1998). In early xenopus embryo, FSTN inhibited the effects of BMP-2 and its receptor (Lemura *et al*., 1998). FSTN can also inhibit MSTN, a member of TGF-β super family which act as a negative regulator of skeletal muscle mass (Lee and Mcpherron *et al*., 2001). Within somites, the expression follistatin was localized to the dorso-lateral part of the somites, which give rise to skeletal muscle of body walls, and limbs (Christ, 1977; Ordahl *et al*., 1992). Follistatin not only function as a activin binding protein, but also interacts with other TGF-β family members through similar binding mechanism. (Otsuka *et al*., 2001a). However, our understanding of variation in the coding regions of FSTN is very limited in chicken. The present study was carried out to determine polymorphism of exon4 of follistatin gene and its association with body weight in PD-1, a broiler line of chicken.

The present work was carried out in PD-1 line, a broiler type line reared at the farm of ICAR- Directorate of Poultry Research, Hyderabad. The PD-1 line was developed from Cornish and its body weight at 6 and 20 weeks of age was 668 and 1986g, respectively (PDP Annual Report, 2012). The birds were reared on deep litter system under intensive management of farming providing *ad-lib* feeding and watering. Chicks were fed with different feeds depending upon stage of the growth. The diet containing 21% and 16% crude proteins were fed to the chicks up to 0 to 3 and 3 to 6 weeks of age, respectively. During the brooding stage, required heating was provided with 100 Walt bulbs. The birds were vaccinated with Marek's disease, Newcastle disease (ND), Infectious Bursal disease (IBD) vaccines at day 1st, 7th and 14th, respectively. The ND and IBD booster of vaccines were given at 14th and 24th day, respectively. Required space of 0.03 to 0.09 m^2 were provided form day1 to 6th week under the deep-litter system. Water sprinklers on roof top were provided to maintain a congenial ambient temperature during the summer season for expressing their optimum potential.

Approximately, 0.1 ml of blood from wing vein of 178 birds of PD-1 line were collected in 1 ml tube containing 2.7% 0.5M EDTA (60-70µl per ml of blood) as anticoagulant. The genomic DNA was isolated from blood cells using standard protocol (Bhattacharya *et al*., 2011). Agarose gel (0.8%) electrophoresis (0.8%) was carried out to check the quality of DNA, and DNA was quantified by Nanodrop spectrophotometer. All DNA stocks were diluted using nuclease free water to produce a standard DNA concentration of 100 µg/µl.

Two pairs of primers were designed from the chicken FSTN sequence available at the National Centre for Biotechnology Information (Accession number- NC_006127) with DNASTAR software (Lasergene Inc). The primer sequences are presented in Table 1. The PCR reactions mix was prepared using 100uM of DNTP mix, 1.5 mM of $MgCl_2$, 10 pM of each primer, 0.3U Taq polymerase and 50µg of DNA template. The temperature used for PCR reaction was, initial denaturation at 94˚C at 5 min, denaturation at 94˚C for 45s for 30 cycles, annealing at 55˚C for 30s, and extension at 72˚C for 45s with final extension at 72˚C for 10 min.

The SSCP involves denaturation of the double–stranded PCR product by heating at 95ºC for 5min (with formamide dye 95% formamide, 0.025% xylenecyanol, 0.025% bromophenol blue, 0.5M EDTA) followed by snap cooling on ice for 15 min. The product was loaded in 12% polyacrylamide gel (PAGE) the gel and electrophoresis was performed at 4ºC for 12h at 200V. After electrophoresis was over, the gel was stained with 0.1% silver nitrate to visualize banding patterns of the fragments, used with modifications (Bhattacharya, 2011; Paswan *et al*., 2014).

Representative sample of different genotypes were sequenced for sequenced by fragments-specific primers from both ends by the automated dye terminator cycle sequencing method in ABI PRIZM 377 (Perkin-Elmer). Sequencing results were aligned as with the sequence of the fragment reported earlier at NCBI sequence with DNAstar software.

PCR amplification of exon4 fragment of FSTN gene revealed the size of 225bp in length. Polymerase chain reaction and Single-Stranded Conformation Polymorphism was used to study structural variation in exon 4 region of FSTN gene. PCR-SSCP revealed that FSTN exon4 was non-polymorphic in all 178 birds of PD-1 chicken population (Fig. 1).

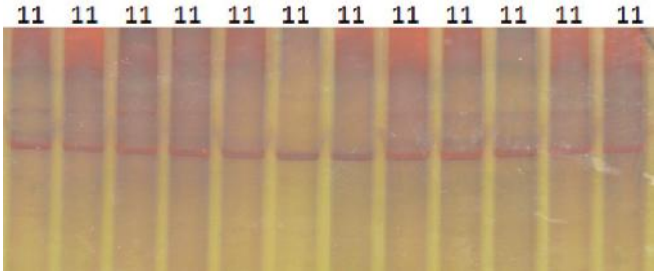

Fig. 1: SSCP patterns of FSTN Exon 4 in PD-1 chicken line

We analyzed the sequence of exon4 in PD-1 line by comparing with the reported sequence (NCBI accession no- NC_006127) of chicken sequence and found that there were transitional type of mutations at 15 G >A and 46 T>C. These mutations were common in all individuals; hence there was no structural variation in FSTN exon4 fragment in PD-1 chicken population. FSTN Exon4 nucleotides were translated into amino acids and compared with the reported sequence (NCBI accession no- NC_006127) to check how mutations at 15th and 46th position of FSTN exon4 affected the coding of amino acid sequence of follistatin protein. Non synonymous mutations were observed where methionine (ATG) was replaced by Isoleucine (ATA) and Tryptophan (TGG) was replaced by Arginine (CGG) at 5th and 16th position of FSTN protein (Fig. 2). This observation was in contrast to one study

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Reference sequence   60   RPVGMFYAQAAPRVWWIKLITPTVHVIEFALSLPPLSSISVGMMALTPVPATEKRPACWA
Our sequence         60   RPVCIFYAQAAPRVWRIKLITPTVHVIEFALSLPPLSSISVGMMALTPVPATEKRPACWA
                          ****:********** ************************************************

Reference sequence   71   DPLDPTRENAS
Our sequence         71   DPLDPTRENAS
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Fig. 2: Alignment of Amino Acid Sequence coded by FSTN Exon4 nucleotide in PD-1 chicken; amino acid bases showing mutation in comparison with reference sequence (NCBI Accession no- NC_006127) were indicated in italic and shaded at highlighted positions

which reported polymorphism in coding region of FSTN gene in humans (Jones, 2007; Sean *et al*., 2007). They also reported different haplotypes of follistatin gene which were associated with skeletal muscle mass in humans.

The body weight of male at day 1, 2nd, 4th and 6th week of age were 40.48±0.37, 138.67±2.16, 438.63±42.50, and 730.41±11.07g, respectively. Weight of females at corresponding age was 40.27±0.34, 135.88±2.21, 380.03±6.04, and 698.21±10.11g. (Table 1). Body weight of male and female did not differ significantly at day 1, 2nd and 4th weeks of age but at 6th week of age body weight differed significantly (p = 0.034) between male and female birds. The body weights of broiler birds were collected at grower stage from day1 to day 42 (Bhattacharya, 2015; Paswan *et al.,* 2014). The body weight observed at different ages in PD-1 line was similar to the earlier reports of (Haunshi *et al.,* 2015).

Table 1: Growth Performance of PD-1 line

Parameters	Male	Female	p-value
Bwt (Day1)	40.48 ± 0.37^a	40.27±0.348^a	0.689
Bwt (2nd week)	138.67 ± 2.16^a	135.88±2.215^a	0.376
Bwt (4th week)	438.63 ± 42.5^a	380.03±6.049^a	0.132
Bwt (6th week)	730.41 ± 11.0^b	698.21±10.11^b	0.034

Bwt (Body Weight), Day1 (Hatch Day), 2nd wk (Second Week), 4th wk (Fourth Week), 6th wk (Sixth Week), Row-wise superscripts with same alphabet indicates non-significant difference and different superscript indicates significant difference along row.

CONCLUSION

Follistatin Exon4 gene was found to be monomorphic in PD-1 chicken population. As compared to reported sequence, two non-synonymous types of mutations were observed in this line. The sex wise differences of body weight observed at 6 weeks of age in this chicken population.

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