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PCR-RFLP OF IGFBP-3 GENE IN MADGYAL SHEEP

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Abstract: Considering the economic importance of Madgyal sheep and close association of IGFBP-3 gene on the growth of mammals, the present investigation was carried out to genotyping of the Madgyal sheep for exon-2 and exon-3 locus of IGFBP-3 gene and to identify polymorphism associated with available data from birth weight in Madgyal sheep using PCR-RFLP techniques. Fifty blood samples of Madgyal sheep collected from Punyashlok Aahilyadevi Sheep and Goat Development Corporation farms at Ranjani and Mahud, were genotyped following DNA isolation by traditional phenol: chloroform: isoamyl alcohol method. A 654 bp fragment of IGFBP-3 gene (comprising of a partial exon 2, complete intron 2, exon 3, and a partial intron 3) was successfully amplified. On digestion of 654 bp of IGFBP-3 gene with HaeIII restriction enzyme yielded single restriction pattern of four fragments of sizes 201 bp, 201 bp, 87 bp and 67 bp in all the animals under study. We could not detect any mutation in the IGFBP-3 (HaeIII) genes of Madgyal sheep. All Madgyal sheep genotyped for IGFBP-3 gene were found to be wild type monomorphic.
Keywords: IGFBP-3 gene; PCR-RFLP; Sheep; Polymorphism

INTRODUCTION

Domestic sheep represents one of the most important livestock species in the world producing meat and wool. Sheep contributes around 12.71 per cent of the total livestock population, comprising of 65.06 million of sheep in India out of which 23.7 lakhs of sheep are found in Maharashtra state [1]. There are 42 documented breeds of sheep in India out of which Deccani and Madgyal are found in Maharashtra. Madgyal sheep derives its name after Madgyal village Tal- Jat Dist-Sangali in Maharashtra and evolved by selective breeding. This breed of sheep is large in body size and sturdy. The colour is predominantly white with brown patches on body. The ram and ewes are polled; have Roman nose (parrot mouth) and ears are long and drooped. The sheep is considered useful for meat and wool production. Comparatively, Madgyal sheep has higher body weight than Deccani from birth weight to twelve month of age. Average body weight at 12 months of age is 45 to 75 kg with an average weight gain of 150-200 gm per day. The annual wool production is 500-560 gm per

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sheep [2]. Therefore, local farmers prefer Madgyal sheep over other breeds for breeding and rearing.

Insulin-like growth factors (IGFs) are the major polypeptides that regulate cell differentiation, growth, metabolism and mitosis in mammals [3,4]. The IGFs stimulate initial mammary gland growth and development and have a galactopoietics role [5,6]. Insulin-like growth factor binding protein-3 (IGFBP-3), which is encoded by the insulin-like growth factor binding protein 3 gene (*IGFBP-3*), is a structural gene responsible for the multiple influences of IGFs system. The IGFs signalling system is an evolutionarily conserved signalling pathway. This signalling system is composed of IGF-I, IGF-II, IGF-I receptor, IGF-II receptor and six binding proteins (IGFBP-1–IGFBP-6), play an important role in development, growth and reproduction as well as ageing [7,8,9]. IGFBP-3 is used as a marker for different body functions such as growth, metabolism, reproduction, in controlling body weight, immunity and energy balance..

The ovine *IGFBP-3* gene is located in the chromosome 4 [10]. The full length of *IGFBP-3* is 8.9 kb with five encoding exons [11]. Due to the key role of *IGFBP-3* in growth and development of animals, the *IGFBP-3* gene is considered as a candidate gene for its use as a marker for growth and production traits. Hence, the polymorphism in *IGFBP-3* gene has been studied in different livestock such as pig, cattle, buffalo, sheep and goat for its association with economic traits. Nucleotide sequences of the *IGFBP-3* have been determined in cattle, buffaloes, sheep and goats however positive association of the *IGFBP-3* genotypes with production traits has been reported only in cattle [12] and gayal [13].

Worldwide there is increasing trend of utilizing Marker Assisted Selection (MAS) as a tool for augmenting the genetic improvement of various livestock species. However, in India MAS has not been fully exploited. It may be due to lack of information about association of candidate genes with the important economic traits. Therefore, considering the importance of Madgyal sheep and *IGFBP-3* gene, present study was undertaken to identify the *IGFBP-3* gene polymorphism and its association with the birth weight in Madgyal sheep. Therefore, the study was designed for genotyping of the Madgyal sheep for *IGFBP-3* gene exon-3 locus (*Hae*III) using PCR-RFLP and to conduct association studies of identified polymorphism with available data from birth weight.

MATERIALSAND METHODS

Sample collection and DNA extraction

The experimental material for present study comprised of 50 blood samples collected from Madgyal sheep maintained at Sheep and Goat farm, Mahud, Dist. Solapur, Ranjani, Dist. Sangli under Punyashlok Aahilyadevi Maharashtra Sheep and Goat Development Corporation Ltd., Pune. The blood samples were collected aseptically in EDTA vacutainer and transported to the laboratory on ice. The samples were stored at 4°C till further processing. The genomic DNA was isolated from blood samples using traditional Phenol:Chloroform:Isoamylalcohol (P:C:I) method as described by [14] with minor modifications. The quantity and quality of DNA was checked by spectrophotometer (NanoDrop ND-2000, Thermo, USA).

***PCR-RFLP of IGFBP-3 gene*:**

To amplify the region of *IGFBP-3* gene one primer set was used as forward (P3: 5′-CCA AGC GTG AGA CAG AAT AC-3′) and reverse (P4: 5′-AGG AGGGAT AGG AGC AAG AT-3′) [15] which was expected to amplify a 654 bp *IGFBP-3* gene fragment comprising of partial exon 2, complete intron 2, complete exon 3 and partial intron 3. For amplification, 25 ul of PCR reaction was prepared by adding 10 pM of each forward and reverse primers, 10 pM of each dNTPs, 10X PCR green buffer, 80-100 ng DNA template and 1 Unit *Taq* DNA polymerase (Thermo Scientific EP0702). The amplification was carried out using a pre-programmed thermal cycler (Eppendorf Master cycler gradient nexus) with the following conditions : PCR thermal cycles for *IGFBP-3* (654bp) gene locus amplification, initial denaturation of 2 min at 94^{o}C followed by 11 cycles of denaturation for 45 sec at 94^{o}C, annealing for 1 min at 62^{o}C and extension of 1 min at 72^{o}C followed by 29 cycles denaturation of 45 sec at 94^{o}C, annealing of 1 min at 55^{o}C and extension of 1 min at 72^{o}C lastly the final extension of 10 min at 72^{o}C. PCR amplification was confirmed by running 5 µl of PCR product from each tube on 1.7 per cent agarose gel. An aliquot of 10µl of PCR product was digested overnight with 1 Units of *Hae*III restriction enzyme (New England BioLabs R0108S). The restriction enzyme digested PCR products were separated by 2.5 per cent agarose gel electrophoresis and stained with ethidium bromide. The digested products were visualized and documented under gel documentation system (UVP, UK).

RESULTS AND DISCUSSION

The present study was carried out to genotype Madgyal Sheep at *IGFBP-3* gene loci using PCR-RFLP method. In the present study locus comprising partial exon 2, complete intron 2,

complete exon 3, partial intron 3 (654 bp) of *IGFBP-3* gene was successfully amplified. The single compact band of 654 bp amplicons of *IGFBP-3* gene are shown in Figure no. 1. Similar results i.e. amplification of sheep *IGFBP-3* at 654 bp were reported by Amr [16] in Egyptian breeds of sheep namely Rahmani, Ossimi, Awassi, and Barki, Sharma [15] in Muzaffarnagari sheep, Shafey [17] in Egyptian sheep breeds (Barki, Rahmani and Osseimi) and Tep [18] in Osmanabadi goats. However, Padma [19] obtained 655 bp PCR fragment in Indian buffaloes while Choudhary [20] observed 651 bp amplicon in Holstein Friesian cattle using same set of primers. Individual PCR products were then subjected for *Hae*III RFLP analysis.

The *Hae*III PCR-RFLP of 654 bp amplicon of *IGFBP-3* gene from the Madgyal sheep revealed only one type of restriction pattern yielding eight fragments of sizes 201 bp, 201 bp, 87 bp, 67 bp, 56 bp, 19 bp, 16 bp, and 7 bp, presented in Figure no. 3. However, two 201 bp bands appeared as a single band while 56, 19, 16 and 7bp were not visible probably due to their small size. Thus, after restriction digestion of 654 bp PCR product three bands of 201 bp, 87 bp and 67 bp were visible in the agarose plate. In the present investigation only AA genotype was observed with respective genotypic frequency as 1. These findings are in complete agreement with Kumar and his coworkers [21].

The *Hae*III PCR-RFLP of *IGFBP-3* gene could not detect any polymorphism in studied Madgyal sheep population. These findings suggest the monomorphic nature of Madgyal IGFBP-3 gene at studied locus. These findings are in agreement with [21, 16, 15 and 17].

Lan [21] reported single restriction pattern with eight fragments of sizes 201, 201, 87, 67, 56, 19, 16 and 7 bp after digestion of 654 bp fragment of *IGFBP-3* gene with *Hae*III restriction enzyme. They revealed absence of polymorphism at given locus in five Indian sheep breeds. Amr [16] also reported the similar results in the four Egyption sheep breeds (Rahmani, Osseimi, Barki and Awassi) indicating the homozygosity of the *IGFBP-3* gene in these breeds. They also reported that all the sheep had intact *Hae*III restriction site (GG ↓CC) indicating the absence of polymorphism at the said locus. Sharma and Shafey [15,17] also confirmed the homozygosity at the IGFBP-3 locus in Muzaffarnagari and three Egyptian sheep breeds, respectively. These results were in accordance with the present findings. Similar findings were reported in five breeds of Indian buffaloes by Padma [19] However, 655 bp amplicon of *IGFBP-3* gene on digestion with *Hae*III yielded slightly different fragments as 201, 165, 154, 56, 36, 19, 16 and 8 bp. In Osmanabadi goat breed also observed monomorphic nature of *IGFBP-3* gene at the said locus [18].

The *IGFBP-3* gene polymorphism has been reported in different species. Choudhary [20] reported polymorphism in the Holstein Friesian cattle for corresponding locus. The polymorphism in the cattle was due to C → A (GG CC to GG AC) transition in intron 2 of the gene at 299th base position of cattle DNA sequence, which alters the *Hae*III restriction site. Lan [22] observed polymorphisms of *IGFBP-3* gene by PCR-SSCP method in Chinese goat breeds, wherein 8 SNPs were identified in the exon 2 of *IGFBP-3* gene indicating the high level of heterozygosity. Gao [23] detected polymorphism of a T → C transition in the exon 2 of *IGFBP-3* gene in 3 breeds of cattle. Xi [13] reported nine single nucleotide polymorphisms (SNPs) located in intron 2 in Gayal (*Bosfrontalis*). Mohamed [24] revealed two genotypes as CT (422, 366 and 56bp) and TT (366 and 56bp) after *IGFBP-3*-XsPI / PCR-RFLP study in three goat breeds (Barki, Damascus and Zaraibi).

In the present research work, *Hae*III PCR-RFLP of *IGFBP-3* gene revealed monomorphic restriction pattern of *IGFBP-3* gene and presence of only one genotype in all Madgyal sheep (n = 50). These findings indicates fixation of IGFBP3 gene in Madgyal sheep. Hence, no association could be established between polymorphic pattern of *IGFBP-3* gene and birth weight.

CONCLUSION

In the present investigation, Madgyal sheep were found to be monomorphic carrying only wild type of allele at *IGFBP-3* gene locus comprising of partial exon 2, complete intron 2, complete exon 3, partial intron 3. Therefore, no association could be established between *IGFBP-3* gene polymorphism and birth weight of Madgyal sheep. Since the study was limited to small number and specific region, the study can be undertaken on large scale in order to genotype the Madgyal sheep in different parts of the breeding tract of these sheep.

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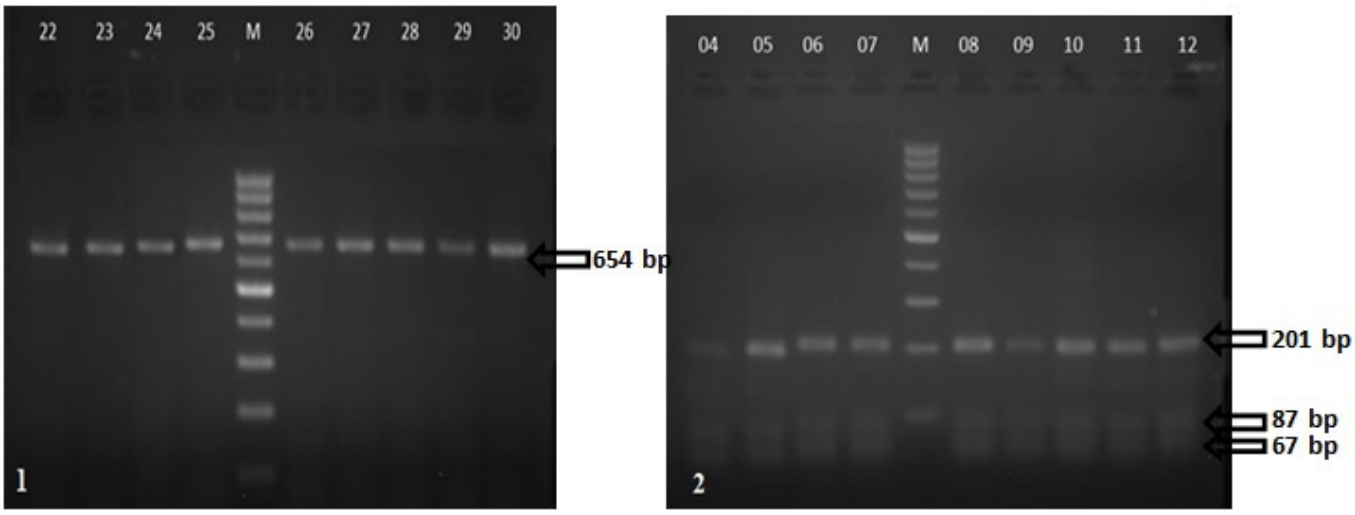

Fig. 1- Agarose gel electrophoresis of IGFBP-3 PCR fragment (654 bp). Lane M: 100 bp DNA ladder, PCR amplification of 654 bp IGFBP-3 gene locus of sample No. 22 to 30 respectively. **Fig. 2-** PCR-RFLP of 654 bp IGFBP-3 gene fragment using *Hae*III Restriction enzyme.Lane M: 100 bp DNA ladder, RE enzyme (*Hae*III) digested products of 654 bp IGFBP-3 gene fragments (201 bp, 87 bp, 67 bp). Sample No. 04 to 12 respectively.

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