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POU1F1 Gene Polymorphism and its Associations with Body Weight of Osmanabadi Goat

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ABSTRACT

Background: Osmanabadi is the premium meat type goat breed of Maharashtra, known for its adaptability and reproductive efficiency. Identification of superior germplasm and incorporating them into breeding programme is the need of hour for augmenting productivity of Osmanabadi as well as other indigenous goat breeds. Hence, the present research was aimed to study Pituitary Specific Transcription Factor I (POU1F1) gene polymorphism and its associations with body weight of Osmanabadi goat.

Methods: Genomic DNA was extracted from 217 blood samples of randomly selected Osmanabadi goats. The 450 bp fragment of POU1F1 gene encompassing exon-6 and its flanking region (3'UTR) was PCR amplified. The PCR amplicon was subjected to RFLP using *Alu*I and *Pst*I restriction enzymes to identify polymorphism at nucleotide position 174 (T>C) in exon 6 and at nucleotide position 110 (T>C) in the 3'UTR of POU1F1 gene, respectively. The association of the observed allelic variants / genotypes with body weight of the animals was performed using One-way ANOVA.

Result: The *Alu*I PCR-RFLP of 450 bp amplicon of POU1F1 gene in Osmanabadi goats revealed polymorphism with three different genotypes *viz*., 'CC', 'TT' and 'TC'. The observed frequencies for TT, TC and CC genotypes were 0.604, 0.355 and 0.041 respectively. The frequencies of T and C alleles were found to be 0.781 and 0.219, respectively. However, *Pst*I PCR-RFLP revealed monomorphic 3'UTR of POU1F1 gene with single TT genotype. We observed significant differences (P<0.01) between various genotypes at *Alu*I exon-6 locus of POU1F1 gene and body weights at birth, 3 and 6 months of Osmanabadi goat. Highest body weight was recorded for TT genotype followed by TC and CC genotypes in all three age groups. Our findings indicate that the *Alu*I PCR-RFLP locus of the exon-6 of POU1F1 gene can be used as a potent molecular marker for selection of superior stock of Osmanabadi goats.

Key words: Osmanabadi goat, POU1F1 gene, PCR-RFLP.

INTRODUCTION

In India, sheep and goat farming has been an important source of livelihood especially for economically weaker section of the society. There are 148.88 million goats which contributes about 19 per cent of total meat production in the country (20th Livestock Census, 2019; www.dahd.nic.in). Out of total 34 registered Indian goat breeds, Osmanabadi is the predominant meat purpose goat breed of Maharashtra. It is popular because of its high prolificacy and adaptability to harsh environmental and primitive managemental conditions. It is a medium-sized breed with comparatively long body and long legs. It's observed average birth weight was about 2.5 kg; while, body weights of adult female and male were 30-35 kg and 45-50 kg, respectively (Deokar *et al.*, 2006). Under organized conditions, it has the breeding efficiency up to 92 per cent with kidding percentage and twinning ability as 55.87 and 10.52%, respectively (Sahare *et al.*, 2009).

Faster growth rate has been recorded in few of the Osmanabadi goats, however, there is a wide variation of growth rate in these goats. The variation might be due to the underlying genetic variation, which can be explored by using advanced molecular techniques. Incorporation of candidate gene approach in conventional breeding programmes would augment the genetic gain and productivity of Osmanabadi goats. The pituitary specific transcription factor 1 (POU1F1) is one of the candidate genes that influences the growth and body mass of the animal, as reported by Supakorn (2009).

Caprine, ovine and bovine POU1F1 gene (also known as pituitary specific transcription factor-1, PIT-1 or growth hormone factor-1, GHF1) is located on chromosome 1q21-22 (Woollard *et al*., 2000). The product of POU1F1 gene, PIT-1 is mainly expressed in the anterior pituitary gland. It regulates expression of GH, PRL, TSH-β genes and itself. It is essential for differentiation, reproduction and survival of somatotropes lactotropes and thyrotropes. The inhibition of POU1F1 synthesis leads to a marked decrease in

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proliferation of cell lines producing PRL and GH (Li *et al*., 1990; Lin *et al*., 1992; Sun *et al*., 2002). The POU1F1 polymorphisms are found to be associated with birth weight, body weight, milk yield, milk proteins, fat yield and litter size mainly in cattle and goats. Hence, considering the potential association of POU1F1 gene variants with growth traits, present research was carried out to study caprine POU1F1 gene polymorphism and its association with the body weights of Osmanabadi breed of goat.

MATERIALS AND METHODS

The present research was carried out at 'Genetic Investigation Laboratory', Department of Animal Genetics and Breeding, Mumbai Veterinary College, Mumbai during the year 2017-2018.

Experimental animals and DNA extraction

The experimental material comprised blood samples collected from randomly selected Osmanabadi goats (n=217) of either sex maintained at various Sheep and Goat farms, of Punyashlok Aahilyadevi Maharashtra Sheep and Goat Development Corporation Ltd., Pune. The birth weight, three-month body weight and six-month body weight data were collected during sample collection. The genomic DNA was isolated from blood samples using traditional Phenol: Chloroform: Isoamyl alcohol method of DNA isolation (Sambrook and Russell, 2001).

PCR amplification

The 450 bp fragment of POU1F1 (exon-6 and its flanking region) was PCR amplified by using the primer pair (F: 5′CCATCATCTCCCTTCTT3′ and R: 5′AATGTACAATGT CCTTCTGAG3′) as described by Lan *et al*., 2009a. The 25 µl final PCR mixture consisted of 1X 'Dreamtaq' green PCR Buffer, 2 mM of $MgCl_2$, 0.2 mM of each dNTPs, 5 pmol of each primer, 1 U Taq polymerase (Thermo-Fisher Scientific) and 1 µl (~50-100 ng) genomic DNA. The PCR cycle was accomplished by initial denaturation for 4 min at 95°C, 35 cycles of denaturation at 94°C for 45 sec, annealing at 55°C for 45 sec. and extension at 72°C for 1 min and the final extension at 72°C for 10 min. The PCR products were visualized on 2.5 per cent agarose gel stained with 1 per cent ethidium bromide.

Restriction digestion of POU1F1 exon-6 and its flanking region

The PCR amplicon was subjected to RFLP using *Alu*I and *Pst*I restriction enzymes to identify polymorphism at nucleotide position 174 (T>C) in exon 6 and at nucleotide position 110 (T>C) in the 3'UTR of POU1F1 gene, respectively. The PCR amplicons (10 µl, ~1 µg) were digested with 10U of restriction enzyme along with 1.5 µl (1X) restriction buffer in a separate reaction.

Statistical analysis

The association of the observed allelic variants / genotypes with body weight of the animals was performed using One-way ANOVA. The linear model used to establish relationship between genotypes and body weights, is as follows;

$$Yij = \mu + \tau i + \epsilon ij$$

Where,

Yij represents the body weight of j^{th} animal with i^{th} genotype, μ is overall mean, τi represents the i^{th} genotype effect and ϵij represents the random error.

RESULTS AND DISCUSSION

The PCR amplification of extracted DNA from goat blood samples yielded a locus specific single band. The designed primers annealed at 55°C and yielded 450 bp amplicon of exon-6 and its flanking region of POU1F1 gene in all samples.

*Alu*I PCR-RFLP of exon-6 and its flanking region of POU1F1 gene

The *Alu*I digested PCR amplicons revealed three different restriction digestion patterns indicating the presence of variation in exon-6 and its flanking region of POU1F1 gene of Osmanabadi goat. The *Alu*I PCR–RFLP revealed three genotypes *viz*., 'CC' (216, 124 and 110 bp); 'TT' (340 and 110 bp) and 'TC' (340, 216, 124 and 110 bp) (Fig 1). The observed frequencies of TT, TC and CC genotypes were calculated as 0.604, 0.355 and 0.041, respectively. The frequencies of T and C alleles were found to be 0.781 and 0.219 in the analyzed population. The *Alu*I PCR-RFLP based polymorphism in POU1F1 gene (exon 6 and its flanking region) was initially observed in native goat breeds of China (Lan *et al*., 2007a). Similar polymorphisms were also

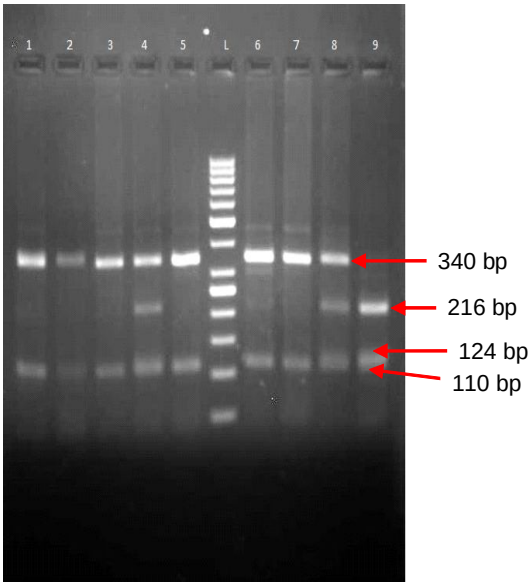

Fig 1: Agarose (2.5%) gel electrophoresis of *Alu*I PCR-RFLP of POU1F1 exon-6 in Osmanabadi goats (lanes 1-9). [Lanes: 1 to 3 and 5 to 7 -TT genotype (340 and 110 bp); Lane 9 - CC genotype (216, 124 and 110 bp); Lanes 4 and 8 - TC genotype (340, 216, 124 and 110 bp); Lane - L 50 bp DNA ladder.

reported in Sarda goats (Daga *et al*., 2013) and Chinese goats (Ma *et al*., 2017).

*Pst*I PCR-RFLP of exon-6 and its flanking region of POU1F1 gene

Restriction digestion of the PCR amplicon with the *Pst*I enzyme revealed a single uncut fragment of 450 bp in all screened Osmanabadi goats (Fig 2). It indicated presence of only one genotype (TT) with a genotypic frequency of 1.0 in the studied population. The expected TC and CC genotypes were not observed in the screened population of Osmanabadi goats. The *Pst*I/ PCR-RFLP assay therefore revealed monomorphic nature of the flanking region of POU1F1 gene in Osmanabadi goats. The monomorphic nature at exon-6 locus of POU1F1 gene was also reported in Barbari goats (Sharma *et al*., 2013). However, *Pst*I polymorphisms were noted in various goat breeds across the world (Daga *et al*.,2013; Lan *et al*., 2009a; Alakilli *et al*., 2012).

Association of exon-6 POU1F1 gene polymorphism with body weight of Osmanabadi goat

Highly significant differences (P<0.01) were observed between the genotypes at exon-6 locus of POU1F1 gene and body weights (birth, 3 and 6 months) of Osmanabadi goat (Table 1). Amongst the three genotypes highest body weight was observed for TT genotype followed by TC and CC genotypes in all three age groups. In case of body weight at birth and six-month of age highly significant (P<0.01) differences were observed between animals with TT and CC genotypes. However, animals with TC and CC did not differ significantly. Animals with TT genotype had significantly (P<0.01) higher body weight at 3 months of age than the TC and CC genotypes; but non-significant differences were observed between the TC and CC genotypes. Thus, present findings suggested that TT genotype has significant association with the body weight at birth, 3 and 6 months of age in Osmanabadi goat, hence it may be considered as a one of molecular marker for selection of Osmanabadi goat.

The POU1F1 gene polymorphism and its association with important economic traits of goat and other species have been reported by various research workers. The TT genotype at *Alu*I/POU1F1 locus recommended as a molecular marker for superior birth weight in Chinese goat by Lan *et al*., 2007a. Similarly, significant positive associations of POU1F1 gene with growth traits in sheep and goats were observed by (Lan *et al*., 2007b; Wang *et al.,* 2013; Ma *et al*., 2017; Dorjay and Abraham., 2021). In addition to growth traits, POU1F1 gene polymorphisms had significant impact on fibre production in Cashmere goats (Lan *et al*., 2009a; Lan *et al*., 2009b); wool traits in Makooei sheep (Negahdary *et al.,* 2014). Further, it was significantly linked with milk production in goat and sheep (Lan *et al*., 2009[c]; Ozmen *et al*., 2014); with litter size in Awassi sheep and Shaanbei White Cashmere goat (Al-Khuzai and Al-Anbari 2018; Zhu *et al*., 2019). The POU1F1 gene mutations also exhibited a positive association with milk protein in cattle (Yan *et al*., 2011); growth and carcass traits of swine (Getmantseva *et al*., 2017).

However, some reports suggested non-significant association of POU1F1 gene with various traits such as dam's body weight and milk production in Jonggol sheep (Sumantri *et al.,* 2009); morphometrical traits of Tianfu goats (JiangZuo, 2010) and birth weight, biometric traits and blood metabolites Iranian sheep (Jalil-Sarghale *et al.,* 2013).

The present research findings indicates that POU1F1 genotypes influences body weights, hence it can be used

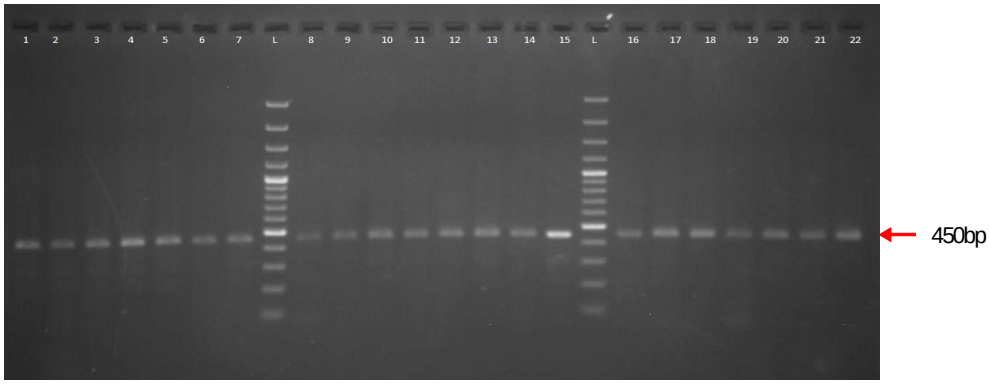

Fig 2: Agarose (2.5%) gel electrophoresis of *Pst*I PCR-RFLP of POU1F1 exon-6 in Osmanabadi goat (lanes 1-22). Lanes 1 to 22 – TT genotype (450 bp); Lane: L, 100 bp DNA ladder.

Table 1: Association of the POU1F1 exon-6 genotypes with body weight of Osmanabadi goat (mean ± S.D.).

Body weight (Kg)	Genotype TT	Genotype TC	Genotype CC
Birth weight	2.404±0.446[a]	2.153±0.477[ab]	1.978±0.390[b]
3-month body weight	8.572±1.600[a]	6.429±1.017[b]	6.111±1.024[b]
6-month body weight	12.317±1.273[a]	11.163±1.488[ab]	11.000±1.118[b]

Body weights significantly different at P<0.01.

as one of the molecular markers for the growth in Osmanabadi goats.

CONCLUSION

The present investigation revealed the polymorphism at nucleotide position 174 (T>C) of POU1F1 (exon 6 and its flanking region) and its significant association with body weight of Osmanabadi goat. The animals with TT genotype had significantly higher body weight hence, it may be considered as one of the potential molecular marker for superior growth in Osmanabadi goat.

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