Prolactin (PRL) is an anterior pituitary peptide hormone which regulates more than 300 activities in vertebrates, including endocrinology and metabolism, brain and behaviour, reproduction, immune regulation and protection (Bole-Feysot et al., 1998; Binart et al., 2010). Its lactotrophic potential is facilitated in combination with its receptor, PRLR is detected in various tissues including brain, ovary, placenta and uterus in several mammalian species. The PRLR gene was mapped to chromosome 20 in goats and 16 in sheep, and is composed of 10 exons (Lu et al., 2011). PRLR is a strong candidate gene for milk production and reproduction (Parihar et al., 2017).

Several PRLR gene polymorphism have been reported in different breeds, including Chinese Liaoning cashmere goat (Zhou et al., 2011), Jining Grey goat (Ran et al., 2011), Xinong

India is a mega-biodiversity, contributing a proportionate share of livestock breeds to the world (Sadana and Pandey, 2004). Goat meat (chevon) is a good source of protein with low fat and cholesterol content, suitable for human consumption (Banskalieva et al., 2000). India has 34 well defined breeds of goats out of which 5 breeds, Surti, Mehsana, Kutchi, Zalawadi and Gohilwadi are native to Gujarat (ICAR-NBAGR, 2021). The Surti and Mehsana goat breeds are kept for both milk and meat purpose which are found in two different parts of Gujarat, Surat and Mehsana, respectively.

Growth traits determine economic worth of the animal. Animal growth is controlled by Hypothalamic-pituitary-somatic (HPS) axis, which is responsible for the secretion of the growth hormone (GH) from the pituitary gland and stimulation of insulin-like growth factor factor 1 (IGF1) from the liver (Wang et al., 2004). The IGF1 gene sequence in goats is located on chromosome 5 (Schibler et al., 1998) and is composed of 6 exons. IGF1 aids in cell differentiation, embryogenesis, reproduction, foetal development, regulation of metabolism and growth (Adam et al., 2000; Shen et al., 2003). Due to the various roles of IGF1 gene in animal growth, it has been considered as a candidate marker associated with growth and carcass traits in various domestic livestock species.

IGF1 gene polymorphism have been reported in various goat and sheep breeds by various researchers including Jamunapari and Sirohi goats (Sharma et al., 2013), Egyptian sheep and goat breeds (Othman et al., 2016), Iranian Markhoz goat (Rasouli et al., 2017), Malabarri and Attappady Black goats (Naicy et al., 2017), Assam Hill goat (Sarmah et al., 2019), Indonesian Kejobong goat (Lestari et al., 2020).

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Saanen, Guanzhong, and Boer goat breeds (Hou et al., 2014), Chinese Hu sheep (Wang et al., 2015), Chinese Haimen goat (Dejun Ji et al., 2016), Gaddi goat (Sankhyan et al., 2019), Shannbei white cashmere goat (Xin Feng Liu et al., 2019).

The present study was undertaken to detect genetic polymorphism in 5’NCR of IGF1 gene and 3’UTR of PRLR gene in Surti and Mehsana goats.

MATERIALS AND METHODS

Ethical Statement: Technical program was approved by Institutional Ethical Committee.

Genomic DNA Extraction

Blood samples were collected from 50 Surti goats maintained at Livestock Research Station, NAU, Navsari and 50 Mehsana goats maintained at Livestock Research Station, SDAU, Sardarkrushinagar, Gujarat, respectively. About 5 mL of the blood was collected from the jugular vein of each animal into sterile 10 mL vacutainer containing 0.5M EDTA as anti-coagulant and stored at -4ºC till further processing. Genomic DNA was extracted from whole blood samples using the standard phenol-chloroform extraction method in the laboratory (Sigma and Himedia Ltd.) as described by John et al. (1991). Purity and DNA concentration was checked using Nanodrop spectrophotometer (ND-2000c) at optical density (OD) 260 nm and 280 nm. The quality of genomic DNA was checked by 0.8% agarose gel electrophoresis at 80 V for 60 min.

Polymerase Chain Reaction (PCR)

Oligo primers specific to caprine IGF1 gene as specified by Othman et al. (2016) and PRLR gene as specified by Hou et al. (2014) were synthesized and supplied by Eurofins Genomics and utilized to amplify the desired fragments in the present study (Table 1).

<table>
<thead>
<tr>
<th>Gene</th>
<th>Primer sequence</th>
<th>Region</th>
<th>PCR product size</th>
</tr>
</thead>
<tbody>
<tr>
<td>IGF1</td>
<td>F-5’TGAGGGGAGCCAATTACAAGGC3’&lt;br&gt;R-5’CCGGGCGATGAAGACACACACAT3’</td>
<td>5’NCR</td>
<td>294 bp</td>
</tr>
<tr>
<td>PRLR</td>
<td>F-5’AGTGAGAGTTATGGAAGGATG3’&lt;br&gt;R-5’AAGTTAAGCAACTGCTT3’</td>
<td>3’UTR</td>
<td>443 bp</td>
</tr>
</tbody>
</table>

PCR was performed in a total reaction volume of 20 µL consisting of 10 µL of 2X master mix (Takara), 2.5 µL genomic DNA (75 ng), 0.8 µL (8 pmole) of each forward and reverse primer and 5.9 µL of nuclease free water.

PCR amplification of 5’NCR of IGF1 gene was carried out with initial denaturation at 94ºC for 5 min which was followed by 30 cycles of denaturation at 94ºC for 30 s, annealing at 64ºC for 15 s and extension at 72ºC for 15 s and final extension at 72ºC for 5 min. Amplification of 3’UTR of PRLR gene consisted of similar protocol with annealing temperature at 54ºC.

PCR products were analysed on 2% agarose gel electrophoresis for 60 min at 80 V. 50 bp DNA ladder (Qiagen) was used as a molecular size marker. The bands were visualized under UV light after staining with ethidium bromide and documented by gel documentation system (Alpha Imager HP).

Restriction Fragment Length Polymorphism (RFLP)

The Restriction Enzymes used in the study were procured from Takara Bio Inc and Genei Laboratories Private Limited. Restriction digestion of the amplified PCR products were carried out in a total reaction volume of 20 µL containing 10 µL PCR products, 2 µL 10X RE buffer, 7.7 µL nuclease free water and 0.3 µL restriction enzyme. Incubation and inactivation protocol of PCR products for restriction enzymatic digestion is presented in Table 2. Digested products were run on 2% agarose gel in 0.5X TBE buffer for 70 min at 80 V, visualized under UV light and photographed by gel documentation system. 50 bp DNA ladder (Qiagen) was used as a molecular size marker.

<table>
<thead>
<tr>
<th>Restriction enzyme</th>
<th>Recognition Site</th>
<th>Sample</th>
<th>Incubation</th>
<th>Inactivation</th>
</tr>
</thead>
<tbody>
<tr>
<td>HaeIII</td>
<td>5’...GTC...3’&lt;br&gt;3’...CGA...5’</td>
<td>IGF1(294 bp)</td>
<td>37º for 1 h</td>
<td>80ºC 10 min</td>
</tr>
<tr>
<td>AluI</td>
<td>5’...AGCT...3’&lt;br&gt;3’...TGA...5’</td>
<td>PRLR(443 bp)</td>
<td>37º for 1 h</td>
<td>80ºC 10 min</td>
</tr>
</tbody>
</table>

RESULTS AND DISCUSSION

On amplification of 5’NCR of IGF1gene and 3’UTR of PRLR gene, PCR products of 294 and 443 bp, respectively were observed in both the breeds (Plates 1, 2, 3 and 4).
Restriction digestion of 294 bp 5'NCR of IGF1 gene with HaeIII revealed only one genotype GG (Plate 5 and 6). The frequency of genotype GG and allele G were observed 1.0 each in both the Surti and Mehsana goats. Therefore, 5'NCR of IGF1 gene was found to be monomorphic in both the breeds of goats. Similar results were obtained for restriction digestion of PRLR gene 443 bp (3'UTR) fragment with AluI showing monomorphic pattern (Plate 7 and 8). Only one genotype CC was found with an allele frequency of 1.0 in both the Surti and Mehsana goats.

The RFLP pattern for 5'NCR of IGF1 gene was not in agreement with Othman et al. (2016) who reported three genotypes; GG (234, 48 and 38 bp); CG (272, 234, 48 and 38 bp) and CC (272 and 48 bp) in Egyptian sheep and goat breeds. The genotype frequencies for GG, CG and CC were 0.55, 0.29 and 0.16, respectively. The allelic frequencies for G and C were 0.70 and 0.30, respectively. Naicy et al. (2017) also reported two genotypes GG (96 bp, 72 bp) and AG (268 bp, 196 bp, 72 bp) in two native goat breeds of Kerala. The genotype frequencies for GG and AG were 0.62 and 0.38, respectively. The allelic frequencies for G and A were 0.81 and 0.19, respectively.

Similarly, the RFLP pattern for 3'UTR of PRLR gene was not in agreement with Hou et al. (2014) who reported two RFLP bands of 443 and 383 bp in three goat breeds of China. They reported two genotypes CC and CT and their genotype frequencies were 0.67 and 0.33, respectively. The allelic frequencies for C and T were 0.83 and 0.17, respectively. Zhou et al. (2011) also reported results in Liaoning cashmere goats of China with two fragments 136 and 26 bp. They reported genotypes CC and CT and the genotype frequencies were 0.86 and 0.14, respectively. The allelic frequencies for C and T were 0.93 and 0.07, respectively.

**Conclusions**

In the present study, regions of IGF1 294 bp (5'NCR) and PRLR 443 bp (3'UTR) successfully amplified with caprine specific
primers. Absence of polymorphism regions of IGF1 (5’NCR) and PRLR (3’UTR) alleles at both the loci was observed in Surti and Mehsana goats breeds. Both these goats breed populations were observed to be fixed for the selected regions of the IGF1 (5’NCR) and PRLR (3’UTR) which reveals substantial conservation of genome organization among the higher vertebrates.

Acknowledgement
Authors are thankful to the College of Veterinary Science & AH, LU, Navsari, for providing necessary financial support and laboratory facilities needed for this study. Authors are also grateful to Research Scientist, LRS, Navsari and Dantiwada, Gujarat, for their help in sample collection, and Department of Animal Biotechnology for providing valuable technical guidance for this work.

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