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Short Communication

Sequence Analysis of Coding Region of Prolactin Gene in Milking Beetal Goats

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MS: Collected and processed blood samples. AA: DNA extraction and primer designing. AN: Application of bioinformatics tools. TH: Experimental designing and data analysis. JK: Manuscript writing and editing. AW: Collection of blood samples and breed selection. SAR: Caring goat breeds and sample collection. PA: Data collection, conduction of experiments. MM: Manuscript writing, editing and statistics. MEB: Supervision

Key words
Prolactin gene, Beetal goat, mammary gland, Sanger method, BioEdit, Single nucleotide polymorphism

ABSTRACT

Prolactin gene is involved in mammary gland development and milk production. This gene is not characterized molecularly in Pakistani goat breed. It was thus aimed to characterize genetic variations in prolactin gene in beetal goat, one of the famous breeds. A total of 20 goats at milking stage with 3-5 years' age were selected. DNA was extracted by salting-out method and amplification of exon 2, 3 and 4 of prolactin gene was done using specific primers. Amplified products were bidirectionally sequenced through Sanger method. BioEdit was used to edit and align sequenced data for detection of single nucleotide polymorphism while Codon Code aligner and MEGA6 softwares were used for molecular analysis and aligned sequence analysis respectively. Exon 2 and 4 were monomorphic with respect to reference sequence but a deletion of a single nucleotide was found in exon 3. This deletion at *EX3*174delA in coding region of Exon 3 resulted in change in the sequence of amino acids and converted Met to stop codon. This may alter protein structure, expression of the gene and may affect protein's stability. This premature creation of stop codon may lead to truncated protein, low yield of milk or reduction in nutritional value of milk.

Goat is an important livestock species with wide range of geographical distribution and great adaptability to its environment. There are more than 25 breeds of goat found in Pakistan (Anonymous, 2011). For better production of meat and milk, beetal goat is considered as best among all other breeds of goat. Moreover, goats are utilized as a model for biomedical studies (Kon *et al*., 2013; Faisal *et al*., 2013; Saeedabadi *et al*., 2018). In this regard, molecular genetics, for the last decade has played an important role in finding individual or candidate genes with considerable effects on the phenomenon of economic importance. This strategy of recognizing polymorphic genes has led to phenotypic variation based on biochemical and physiological evidence that may also result in speed up the improvement of reproductive trait of goat (Ahlawat *et al*., 2015). Genetic variation in economic traits such as milk, growth, reproduction and meat production at candidate genes level has increased research interest because of considerable support in marking evolutionary relationship and genetic selection in different breeds of livestock (Sodhi *et al*., 2007). Prolactin and myostatin are significant potential genes owing to their positive effect on meat quality characteristics and growth enhancement (Ahlawat *et al*., 2015; Sodhi *et al*., 2007). Polymorphism in sheep and goat prolactin in relation to the wool and cashmere trait has already been reported earlier (Rose *et al*., 1998; Lincoln, 1990; Moreno and Gonzalez, 2004). Additionally, the association polymorphism in prolactin with dairy characters like yield and production of protein milk in cattle breeds has also been reported (Brym *et al*.,

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2005; Alipanah *et al*., 2007; Dong *et al*., 2013). Prolactin is one of the most significant anterior pituitary polypeptide hormones secreted and synthesized by anterior pituitary gland. It plays a significant role in the galactopoiesis, mammogenesis, lactogenesis (Horseman *et al*., 1997; Patel *et al*., 2017). The silent adenosine-guanine (A→G) transition mutation for amino acid (103) in exons 3 of bovine prolactin gene has been reported to increase a polymorphic site for *Rsa*I restrictions enzyme (Lewin *et al*., 1992). It has gained popularity as a genetic indicator used for the genetic classification of *Bos indicus* cattle populations (Sodhi *et al*., 2011; Kumari *et al*., 2008; Sacravarty *et al*., 2008). Keeping in view the above stated facts, present study was planned to analyse coding sequence of prolactin genes in Beetal goats for identification of quality gene for the production of high milk yield in Beetal goat breed, to know about the diversity in the coding sequence of prolactin gene and the effect of variations on milk yield in Beetal goat.

Materials and methods

The blood samples of Beetal goat breed were collected from Directorate of Livestock Farm, Tehsil Jahania District Khanewal, Punjab, Pakistan. A total of 20 healthy, adult milking goats were selected randomly for blood sampling from the stock. These animals were selected on the basis of age (3–5 years), with a history of multiple births to investigate the nucleotide sequences of prolactin gene.

Five mL blood sample was collected from jugular vein of each selected goat in15 mL falcon tube containing EDTA. For DNA extraction standard salting-out method was used as described earlier (Miller *et al*., 1988). To know about the quality and quantitative measurement of DNA, 1.5% agarose gel and NanoDrop spectrophotometer were used, respectively.

The primers for Exon 2 (384 bp), exon 3 (418 bp) and exon 4 (438 bp) of goat-prolactin were designed using primer3 software. The goat PRL gene was amplified using these primers (Table I). The reaction was carried out through 35 cycle that consisted of denaturation at 95 ºC for 45 sec, annealing at respective temperature specified for each exon for 45 sec and extension at 72ºC for 45 sec. Initial denaturation was made at 95ºC for 5 min while final extension was done at 72ºC for 10 min. The reaction volume of 25.0µL was prepared that contained 100-150 ng of extracted DNA, 5pmol of each primer, 245 µL of each dNTP, 1 unit of Taq polymerase and 1x Taq reaction buffer. Gel electrophoresis of the PCR products was done on 2.5% agarose gel possessing ethidium bromide for visualization. The amplified products were processed through ethanol precipitation method and bidirectional sequenced using direct Sanger method (Sanger *et al*., 1977).

The Sequencing results were edited and aligned by BioEdit software for the detection of SNPs in the DNA sequence of goat *PRL* gene. The edited and aligned sequences were analyzed by Codon Code aligner and MEGA6 software for Molecular analysis.

Results and discussion

Sequences of Exon 2, 3 and 4 were analyzed using nucleotide blast and it was found that there was no polymorphism in Exon 2 and 4 (Fig. 1) with respect to reference sequence while a deletion was found in the Exon 3 of prolactin gene of Beetal Goat. The deletion "A" in the Exon 3 did not cause any alteration in the genetic code but it did cause alteration in next codon that changed from Met > Stop codon, thus led to varied sequence of amino acids and hence its effect on production of milk in the Beetal goat.

Goat is an important livestock species of family Bovidae which plays an important role in milk, meat, hides and fibre (Lebbie, 2004; Shrestha, 2005). Among all the breeds, Beetal goat is regarded as best for the production of meat and milk (Faisal et al., 2013; Saeedabadi et al., 2018). The important role of molecular genetics in finding out candidate genes with considerable economic significance, phenotypic variation, physiological, biochemical characteristics and polymorphism in genes

Table I. The primers used for amplification of exon 2, 3 and 4 of prolactin (PRL) gene of goat, with sequences both forward (F) and reverse (R) primers, annealing temperature and product size.

PRL exon	Primer sequence 5`→3`	Tm temperature	Product size
Exon-2	F: AAATCCTGCTCAGGGCAAC R: GCTGATGCAGCCTTCTTGTT	60.3ºC	384 bp
Exon-3	F: GACAAGCAACTGTTTTCAGAGA R: CTTCACCTTTCTCTGCATGTC	57ºC	418 bp
Exon-4	F: ATATAGGGGAAACCTGGAGT R: CTTGCTTTGTGTAATACCAGTC	54 ºC	438 bp

PRL, prolactin

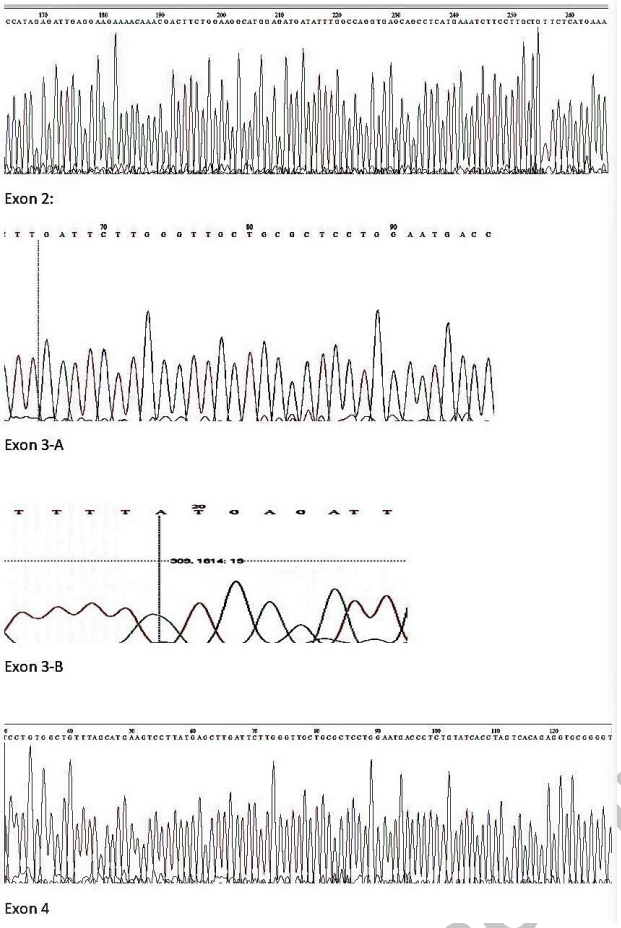

Fig. 1. Nucleotide blast results of Exon 2, 3 and 4 of prolactin gene, a deletion of nucleotide A in Exon.

was recognized which can be helpful for identifying reproductive trait and improvement in milk content in goat as well. Prolactin is a polypeptide hormone secreted and synthesized by anterior pituitary gland and this is the one of most versatile hormones of the pituitary gland in terms of biological activities (Hallerman et al., 1988). Primarily it is responsible for synthesis of lipids, lactose and other main components of milk (Patel et al., 2017; Shamsalddini et al., 2016). So, the gene encoding prolactin is recommended as one of the most significant key links in the gene network. These features make prolactin gene a strong individual gene for milk trait. It was investigated that prolactin gene is a potential genetic marker that supports the performance of cashmere in selection (Shamsalddini *et al*., 2016). A relationship between polymorphism in the prolactin gene of Raini cashmere goats and fibre properties have also been reported. Based on RsaI polymorphism, two allelic variants (B and b) in the coding region of exon 3 have previously been described (Patel *et al*., 2017). These variants were proposed to be associated with milk production. Such markers can hence be used to find out the possible relationship between prolactin gene variants and milk performance characteristics. The polymorphisms in translation and coding regions of bovine prolactin gene of the Chios sheep were also observed (Di *et al*., 2011). Coupling analysis revealed that SNPs have significant effect on milk's fat content and total lactation (Shamsalddini *et al*., 2016). PRL and growth hormone receptors are homologous to receptors of cytokine superfamily. Polymorphisms of intron 1 and intron 2 of prolactin receptor gene were detected in goats with high growth (Jining Gray) and low growth characteristics respectively (Goat Boer, Wendeng Dairy, Liaoning Cashmere and Beijing) (Di *et al*., 2011). However, the genetic variation of intron 1 had no significant effect on the size of the offspring, but intron 2 had a significant effect on the size of the offspring in goat. During this study, exon 2, 3 and 4 of prolactin gene in Beetal goat were characterized for detection of polymorphism and their impact on the production of milk. The exon 2 and 4 were found monomorphic with respect to the reference sequence while a deletion of a single nucleotide was found in Exon 3. This deletion in the coding region of Exon 3 caused the change in the codon sequence of amino acids and resulted in the conversion of Methionine to stop codon. This change in the coding region may alter the protein structure or may alter the process of gene expression, may modify the proteins activity or may affect the stability of protein. This premature creation of stop codon may lead to the truncated protein product leading to low yield of milk or reduction in the nutritional value of milk. Thus, for better understanding, studies with increased number of breeds are suggested.

Conclusion

From the result it has been concluded that the prolactin gene is one of the major contributor in determining the fate of quality of milk and its production.

IRB approval and ethical approval

Current study was approved by Ethical committee of Virtual University ERB (vide letter no. 215/mb/vu dated 09/02/2021).

Statement of conflict of interest

The authors have declared no conflict of interest.

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