Journal of Animal Research: v.5 n.4, p. 253-255. December 2015

DOI: 10.5958/2277-940X.2015.00159.X

SHORT COMMUNICATION

Status of FecB Gene Mutation in Dorper Sheep from Jammu

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Received: 24 September, 2015 **Accepted**: 25 November, 2015

ABSTRACT

The present study was carried out to explore polymorphism in exon-8 of *FecB* gene using PCR-RFLP technique in Dorper sheep. Animals maintained at Government Sheep Breeding Farm, Panthal, Jammu were studied. Genomic DNA was extracted from blood samples using Phenol-Chloroform-Isoamyl method. The PCR product of 140 bp was amplified using reported primers (part of exon-8 of FecB gene). PCR products was digested for the presence of *FecB* mutation using *AvaII* restriction enzyme. The studied samples were monomorphic and were having only one genotype (*FecB*$^{++}$). Our study reveals that Dorper sheep population in the present study is homozygous and non-carrier of *FecB* mutation.

Keywords: Dorper sheep, *FecB* gene, PCR-RFLP, Polymorphism.

Mostly Indian sheep breeds give birth to one lamb per lambing except Garole sheep which is renowned for high reproductive efficiency and prolificacy. The probable reason for this high prolificacy is due to presence of a single nucleotide mutation in *Booroola fecundity* (*FecB*) gene. The *Booroola* gene helps to add a high level of prolificacy to sheep that fit the environment well, without having to add undesirable traits of another breed. There is a need to conduct a systematic research to evaluate the significance for uniqueness or possession of high fecundity gene or gene combination in various breeds of sheep in India.*FecB* polymorphism can be detected by PCR-RFLP technique. Therefore, the present study was undertaken to ascertain the status of *FecB* gene mutation in Dorper sheep.

The present study was carried out on Dorper sheep maintained at Govt. Sheep Breeding Farm, Panthal, Jammu. A total of 50 random animals were taken for study. Genomic DNA was isolated from the venous blood samples by Phenol-chloroform-isoamyl method (Sambrook and Russell, 2001). Horizontal submarine agarose gel electrophoresis was performed to check the quality of genomic DNA by using 0.8% agarose. A pair of primers for amplification of *FecB* gene were used on the basis of the published sequences as reported by Wilson *et al*. (2001). The forward primer was of 23 bp (5′-GTCGCTATGGGGAAGTTTGGATG -3′) and the reverse primer was of 31 base pair (5′-CAAGATGTTTTCATGCCTCATCAACACGGTC -3′). PCR was carried using the following reaction conditions: initial denaturation at 95°C for 4 min., cyclic denaturation at 94°C for 30 sec., annealing at 60°C for 30 sec. and extension at 72°C for 30 sec. and final extension at 72°C for 5 min. To verify the amplified product, 0.8% agarose gel containing ethidium bromide @ 3µl/100 ml of agarose gel was used. A 100 bp DNA ladder was used to know the size of the amplicon. The amplicon was visualized under UV transilluminator and documented by gel documentation system. The PCR product was digested with restriction enzyme *AvaII* (*Anabaena*

variabilis). The restriction digestion was carried out in 30µl volume. The PCR product was digested with *AvaII* at 37 ºC for 2 hrs. The restriction fragments were resolved in 2.5% agarose gel and visualized in gel documentation system. The band patterns were scored to assign specific genotype.

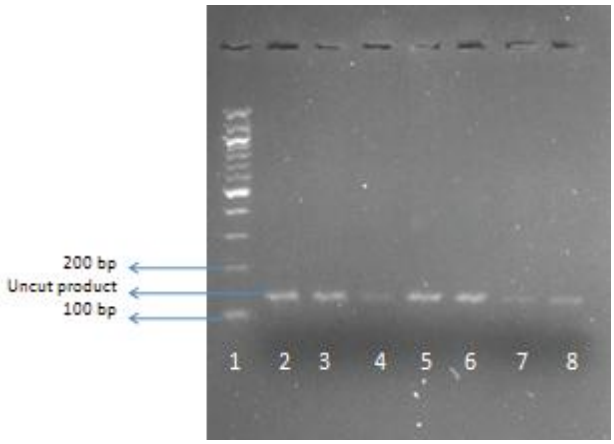

Fig. 1: PCR-RFLP analysis on 2.5% agarose gel electrophoresis of FecB gene in Dorper sheep

Lane-1 -100bp DNA ladder
Lane-2 - 8 - 140bp PCR-RFLP product)

A 140bp size amplicon was obtained as a result of PCR. The amplicon was subjected to RFLP analysis using restriction enzyme *AvaII* which recognizes the site of G/GACC locus and cleaves the DNA fragment. Agarose based genotyping reveals that those animals are $FecB^{BB}$ (Homozygous carrier) shows single 110bp band and those animals are $FecB^{B+}$ (Heterozygous carrier) showed 140bp and 110bp bands. However, those possessing the $FecB^{++}$ genotype (Homozygous non-carrier) reveals an uncut band of 140 bp. In our study, all the individuals revealed a band of 140 bp. Therefore, *AvaII* digestion of PCR amplicons revealed only one type of genotypic i.e. all the animals were homozygous non-carrier type of $FecB^{++}$ genotype.

Since, all the samples were monomorphic for *FecB* gene mutation in Dorper sheep. Hence, genotype frequency of $FecB^{++}$ is reported to be100% and only one type of allele was present in the population i.e. Fec^{+} allele with allelic frequency of was 1.0 whereas Fec^{B} allele was found to be absent.

Our results are in agreement of Amr *et al.* (2009) who reported that the digestion of 190 base pair PCR product (*FecB* gene) with *AvaII* restriction enzyme resulted in non-carrier 190 bp band (wild type) in all the animals belonging to the five Egyptian breeds studied revealing absence of this restriction site in those five Egyptian sheep breed. Similarly, Abulyazid *et al.* (2011) reported that the absence of restriction site for *AvaII* enzyme in Egyptian sheep.

According to Yousif *et al.*(2013) five different Iraqi sheep breeds studied for the presence of *FecB* mutation, using restriction enzyme digestion, were also lacking the mutation for prolificacy. However, Jamshidi *et al.* (2013) reported that the PCR product (*FecB* gene) of Sangsari breed was digested by the short acting enzyme *AvaII* and the existence of the wild monomorphic genotype (++) of the Booroola gene in the flock was observed, indicating a lack of mutation in the examined samples. Moreover, Wan-Somarny *et al.* (2013) reported that the *FecB* and *FecX* genes were successfully amplifiedin Malin and Dorper sheep, however, nomutation occurred in these genes. The results indicated that the *FecB* and *FecX* genes in Malin and Dorper sheep were not polymorphic and thus, can be considered as low prolificacy sheep breeds.

CONCLUSION

Exons-8 region of *FecB* gene of Dorper sheep is monomorphic and only one type of allele i.e. $FecB^{+}$ is present in the studied population. It can be concluded that the reports of twining in Dorper sheep may not be directly related to the mutation of *FecB* gene. Further study/research on large number of Dorper sheep for booroola gene as well as other fecundity genes along with association analysis with lit er size will be help in understanding the underline genetic and physiological basis of twining/fecundity/prolificacy reported in Dorper sheep.

ACKNOWLEDGEMENTS

Authors are thankful to the in-charge and staff of Government Sheep Breeding Farm Panthal, Reasi, J&K, for providing facilities and help for the present study.

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