2011 2nd International Conference on Environmental Science and Development
IPCBEE vol.4 (2011) © (2011) IACSIT Press, Singapore

Genotypic Frequency of Calpastatin Gene in Lori Sheep By PCR-RFLP Method

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***Abstract*—The effect of Calpains gene polymorphism on the analyzed meat quality traits are discussed in detail in another paper. Calpastatin is a natural occurring inhibitor of calpains and consequently the balance of calpain–calpastatin activity in muscles is believed to dictate the rate of tenderization in post-mortem meat. In this study were collected blood samples from 100 Lori sheep. Genomic DNA was extracted from blood sample. Gel monitoring and spectrophotometer methods were used to determination quality and quantity of DNA. MspI enzyme was used for restricting of PCR products. Digested products were separated by electrophoresis on 2% agarose gel and visualized after staining with ethidium bromide on UV transillumination. The PCR product (622 bp) was digested by restriction endonucleases MspI. The MspI digestion of the PCR products produced digestion fragments of 336 bp and 286 bp. Data analysis was done using PopGen32 software (ver.1.32). In the total population of sheep was detected homozygous genotype AA, heterozygous genotype AB and Homozygous genotype BB has been observed for calpastatin gene in Lori sheep strain.**

Keywords- Calpastatin gene, polymorphism, Lori Sheep

I. INTRODUCTION

Calpastatin is present in all tissues expressing calpains and in skeletal muscle. Calpastatin is expressed at a higher level of activity then the calpains themselves. Of the five domains, the N-terminal leader (L) domain does not appear to have any calpains inhibitory activity, but maybe involved in targeting or intracellular localization (Takano et al. 1999), while the other domains (I-IV) are highly homologous and are each independently capable of inhibiting calpains (Cong et al., 1998). This Indicates that the inhibitory domains of calpastatin contain three highly conserved regions, A, B and C, of which A, played a regulatory role by altering phosphorylation patterns on the protein (Takano et al., 1999). The aim of the present investigation was to analyses the polymorphism of the CAST gene in Lori sheep and evaluates its association with daily weight gain traits and C, bind calpain in a strictly Ca^{2+}-dependent manner but have no inhibitory activity, whereas region B inhibits calpain on its own. It is also found that the removal of the XL domain.

II. MATERIAL AND METHOD

Random blood samples were collected from 100 Lori sheep from Lorestan province of Iran. Approximately, 5 ml blood sample was gathered from vena in EDTA tube and was transferred to -20^{o}C freezer. Genomic DNA was isolated by using DNA Extraction Kit and was based on Boom et al. (1989) method. Exon and intron region from a portion of the first repetitive domain of the ovine calpastatin gene were amplified to a product of 622 bp using primers based on the sequence of the bovine (Killefer and Koohmaraie, 1994; Gen bank accession no L14450) and ovine calpastatin genes. Spectrophotometer was used for investigating quality and quantity of DNA. The polymerase chain reaction (PCR) was performed using a buffer PCR 1X, 200 µM dNTPs, 1.5 µM $MgCL_2$, 10 pmol each primer, 1.25 U Taq DNA polymerase, 50 ng ovine gnomic DNA and H_2O up to a total volume of 25 µl. 33 cycle of preliminary denaturation at 95^{o}C (5 min), denaturation at 94°C (1 min), annealing at 60°C (1 min), extension at 72°C (2 min) and final extension at 72°C (8 min). The PCR products were separated by 2% (w/v) agarose gel electrophoresis. The amplified fragment of calpastatin was digested with *Msp*I. 15 µl of PCR production with 2 µl buffer, 5U (0.5) of *Msp*I and 11.5 µl H_2O up to a total volume of 29 µl, followed the manufacturers instruction for 12-16 h at 37°C. The digestion products were electrophoresed on 2% agarose gel in 1X TBE and visualized by ethidium bromide staining for 1 h at 85 V. Estimates genotype and alleles frequencies and Hardy-Weinberg equilibrium was analysis with Pop Gene 32 package (Yeh et al., 1999).

III. RESULTS AND DISCUSSION

The amplified calpastatin resulted in a DNA fragment with 622 bp including the sequences of Exon and intron regions from a portion with PCR technique (Figure1). Within the analyzed, two alleles (A and B) were observed, resulting in three genotypes. The MspI digestion of the PCR products produced digestion fragments of 336 bp and 286 bp (Figure2). Data analysis was done using PopGen32 software (ver.1.32). In the total population of sheep was detected homozygous genotype AA, heterozygous genotype AB and Homozygous genotype BB has been observed for calpastatin gene in Lori sheep strain. This result shows that the polymorphism was detected in CAST I segment, as previously observed by Palmer et al. (1998) and Chung et al. (2001). A and B allele frequencies were 0.638 and 0.362, respectively. The genotype frequencies were 0.407 for AA, 0.131 for BB and 0.462 for AB. The sheep populations were not in Hardy-Weinberg equilibrium and it was concluded that breeding based on selection for Calpastatin gene was done.

ACKNOWLEDGMENT

The current study was supported by The Animal Science Research Institute, Karaj, Iran & Broujerd Branch, Islamic Azad University, Broujerd, Lorestan, Iran. However, we are grateful of Islamic Azad University Branch Broujerd who helps us in this study.

REFERENCES

[1] S. Barnoy , T. Glaser, NS.Kosower (1997). calpain and calpastatin in myoblast differentiation and fusion effects of inhibitors. Biochim. Biophys.Acta. 1358: 181-188.

[2] M. Cong, VF .Thompson, DE .Goll, (1998). The bovine calpastatin gene promoter and a new N-terminal region of the protein are targets for cAMP dependent protein kinas activity. J. Biol. Chem. 273: 660-666.

[3] NE .Forsberg, MA .Ilian , A. Ali-Bar, PR. Cheeke (1989). Effects of cimaterol on rabbit growth and myofibrillar protein degradation and on calcium dependent proteinase and calpastatin activities in skeletal muscle. J. Anim. Sci. 67: 3313-3321.

[4] DE.Goll, VF. Thompson, RG.Taylor, A.Ouali (1998). The calpain system and skeletal muscle growth. Canadian J. Animal Sci. 78: 503-512.

[5] DE. Goll, VF.Thompson , RG.Taylor,T. Zaleweska (1992). Is calpain activity regulated by membranes and autolysis or by calcium and calpastatin . Bio. Essays, 14 (8): 549-556.

[6] E.Huff-Lonergan, T.Mitsuhashi, DD.Beekman, FC.Parrish, DG.Olson, RM.Robson (1996). Proteolysis of specific muscle structural proteins by m-calpain at low pH and temperature is similar to degradation in postmortem bovine muscle. J. Anim. Sci. 74: 993-1008.

[7] J. Killefer, M.Koohmaraie (1994). Bovine skeletal muscle calpastatin: cloning, sequence analysis, and steady-state mRNA expression. J. Anim. Sci. 72: 606-620.

[8] J. Kuryl, W. Kapelanski, M.Pierzchala ,S. Grajewska, M.Bocian (2003). preliminary observation on the effect of calpastation gene (CAST) polymorphism on carcass traits in pigs. J. Anim. Sci. 21: 87-95.

[9] M. Mohammadi, M. T. Beigi Nasiri, K h. Alami-Saeid, J. Fayazi, M. Mamoee and A. S. Sadr.2008. Polymorphism of calpastatin gene in Arabic sheep using PCR- RFLP African Journal of Biotechnology Vol. 7 (15), pp. 2682-2684.

[10] J. Takano, T. Kawamura, M. Murase, K. Hitomi, M. Maki (1999). Structure of mouse calpastatin isoforms: implications of species-common and species-specific alternative splicing. Biochem. Biophys Res Commun. 260: 339-345.

[11] F. Yeh , C. Yang ,T. Boyle (1999). POPGENE version 1.31 Microsoft window-based freeware for Population Genetic Analysis, University of Alberta. Edmonton, AB. Canada.

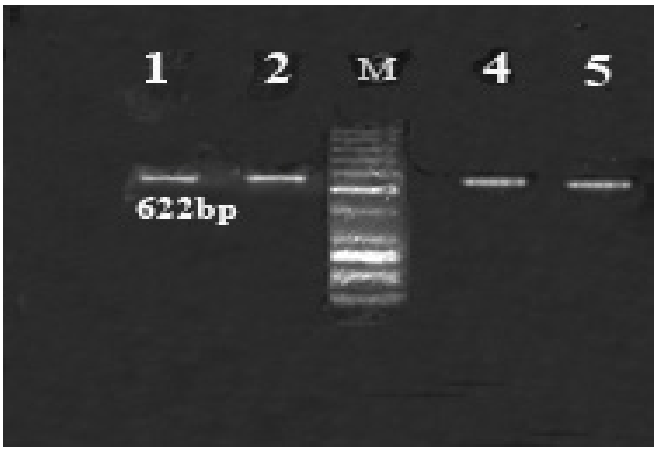

Figure 1. : PCR product analyzed by electrophoresis (622 bp).

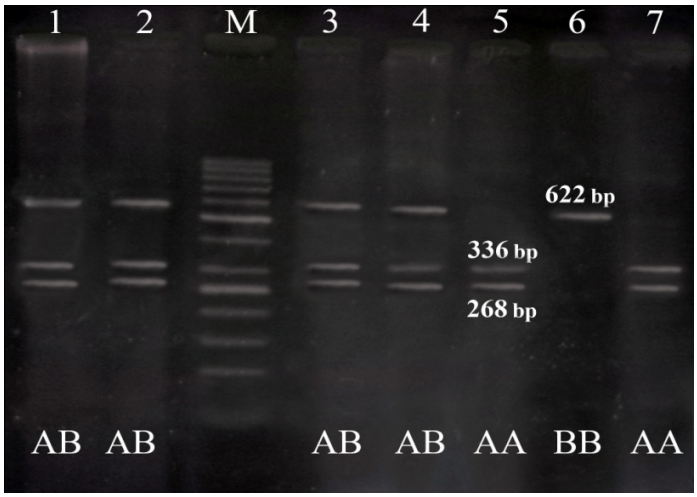

Figure 2. Genotype AA, AB and BB digestion with MspI on 2% agarose gel.