IJAS Iranian Journal of Applied Animal Science

Calpastatin Gene Polymorphism in Raini and Tali Goat in the Kerman Province

Research Article

V. Bahrampour[1*] and A. Mohammadi[2]

[1] Department of Animal Science, Technical and Vocational University, Kerman, Iran
[2] Department of Animal Science, Faculty of Agriculture, University of Zabol, Zabol, Iran

Received on: 4 Jan 2017
Revised on: 20 Mar 2017
Accepted on: 31 Mar 2017
Online Published on: Sep 2017

*Correspondence E-mail: v_bahrampur@yahoo.com

Online version is available on: www.ijas.ir

ABSTRACT

Restriction fragment length polymorphisms (RFLP) have been identified at the goat. The objective of the present study was to determine polymorphisms of calpastatin locus in Raini and Tali goats of the goats in Kerman Province, improving meat quality traits superior meat quality by selection. Calpastatin gene effect on quality and tenderness meat, to identify different genotype of this gene was randomly selected 150 Tali and 150 Raini goats, and blood samples were collected from total of animals using ethylenediaminetetraacetic acid (EDTA) tubes. DNA was extracted from blood according to DNA preparation kit to determine the genetic relationship among studied goat animals. Amplification was performed using polymerase chain reaction (PCR). Two alleles (A and B) and three genotypes, (AA, BB and AB) were observed. The frequencies of the observed genotypes for Tali and raini were 38.4, 9.6, 52 and 17, 71, 12 for AA, BB and AB, respectively. And allele frequencies were 0.47, 0.53 and 0.12, 0.88 for A and B, in Tali and Raini goats respectively.

KEY WORDS calpastatin gene, genotype, PCR RFLP, Raeini goat, Tali goat.

INTRODUCTION

Goat (*Capra aegagrus*), is one of the livestock, first domesticated in Asia. Goats live in different areas. Goat is one of the most adaptable animals with different environmental conditions. Calpastatin, initially identified in skeletal muscle (Busch *et al*. 1972), is the endogenous inhibitor of the calpains (Goll *et al*. 2003). Calpastatins are rich in proline and glutamate, but poor in aromatic amino acids (Murachi, 1983). The rate of protein degradation post mortem affects meat quality (Koohmaraie *et al*. 2002). The calpastatin gene has also been found to have a large effect on pork quality (Rohrer *et al*. 2012). Genetic variation in calpastatin gene and the effect on meat quality traits both in cattle and sheep has been reported by many researchers (Zhou and Hickford, 2008). At the molecular level, calpastatin protein is composed of five-domains having molecular weight of 76 kDa which bind and inhibit the calpains system. The function of N terminal domain is to enhance the targeting of the inhibitory region of calpain system by other inhibitory domain but itself don't play any inhibitory role (Averna *et al*. 2001). Marker assisted selection is one of the new DNA based methods that improves accuracy and progress in animal selection programs (Bastos *et al*. 2001). Genetic markers are important for the determination of allelic polymorphism at any specific locus. A two-allele system of polymorphic variants (M and N) of the ovine calpastatin gene by a PCR-RFLP method has been described (Palmer *et al*. 1998). Tenderness is one of the attributes of quality of beef that is most appreciated by consumers (Alfnes *et al*. 2008). PCR-RFLP is one of the most commonly used methods for polymorphism genotyping due to its simplicity. Initially,

the RFLP analysis required a radio-actively labeled probe for detection, and now the method is coupled with PCR and simple agarose gel electrophoresis. In a study two allels (M and N) determined in region of the ovin calpastatin by PCR-RFLP method (Palmer *et al*. 1999).

MATERIALS AND METHODS

In this study, 150 Tali and 150 Raeni goats was sampled in the Kerman province. Blood samples were taken mainly from the jugular vein. In tubes containing EDTA and immediately transferred to ice, and the samples were stored at -20 ˚C until DNA extraction. DNA was extracted from 0.3 mL blood using the DNA purification kit (Fermentas, EU) according to manufacturer's instructions. Quality and quantity of DNA was measured by agarose gel (2%). The DNA amplification of the calpastatin gene was achieved by PCR. PCR-RFLP genotyping was used to detect the polymorphism in the region between exons 6 and 7 of the calpastatin gene. One pair of primers was used as follows.

Forward primer: 5'-AGCAGCCACCATCAGAGAAA-3'
Reversed primer: 5'-TCAGCTGGTTCGGCAGAT-3'

The final volume was 25 µL reactions, thermal program included: first denaturation temperature of 33 stages with 94 to 30 seconds, 56 ˚C connection temperature for 30 seconds, temperature 72 ˚C for 30 seconds and develop the final amplificon temperature of 72 ˚C for 4 minutes. The correctness of the resulting part of product on 1.5% agarose gel and noticeable with ethidium bromide voltage 82 and was established for 25 minutes.

The PCR products by restriction endonucleases XmnI were digested. Cut pieces again on 2% agarose gel and stained with ethidium bromide voltage 72, after 2 hours determined genotypes. Gel documentation system was used for Photography.

Statistical analysis

For determine gene, genotypic frequency and Hardy-Weinberg equilibrium use Pop-Gene32 program, version 1.31, Canada (Yeh *et al*. 1997).

RESULTS AND DISCUSSION

The samples of PCR showed on the gel agarose that was area between 1500 and 1600 bp. PCR product without any nonspecific band (Figure 1).

The allelic variation in the calpastatin gene was tested by PCR-RFLP. The PCR-RFLP method revealed two alleles; allele B was the not digested and had 1552 bp and allele A was the PCR product with the restriction site for XmnI which upon digestion produced two fragments of roughly 960 and 592 bp PCR product (Figure 2).

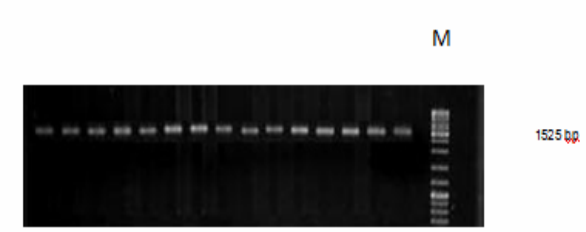

Figure 1 PCR products for calpastatin gene in Raini and Tali goats
Line M is the 100 bp molecular weight marker

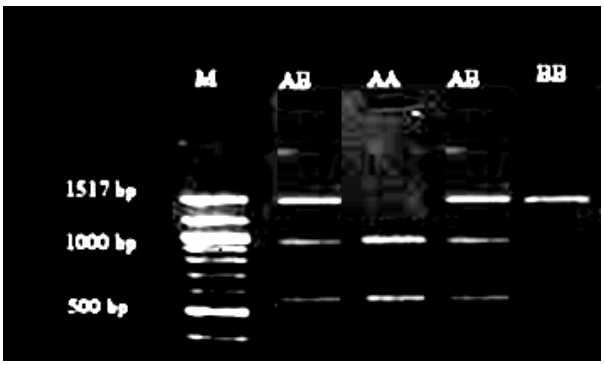

Figure 2 RFLP polymorphism of caprine CAST gene
M shows the 100 bp molecular weight marker
Three different PCR-RFLP patterns (genotype) were identified (AA, AB and BB)

In this study, a total of three genotypes were observed in the test population. The frequencies of the observed genotypes to Tali goat were 38.4, 9.6 and 52.0 for AA, BB and AB, respectively. Allele frequencies were 0.47 and 0.53 for A and B, and for Raini goat also genotypes were 0.17, 0.71 and 0.11 for AA, BB and AB respectively. Allele frequencies were 0.12 and 0.88 for A and B (Table 1).

The chi-square test showed significant ($P<0.01$) deviation from the Hardy-Weinberg equilibrium for this locus in the investigated these two populations. In the present study two alleles (A and B) and three genotypes (AA, BB and AB) were observed for calpastatin gene in Tali and Raini goat in kerman province, Iran.

The most frequent allele and genotype in the Tali goat were 53.0 and 67.0% for allele A and allele B, respectively (Table 2) and to Raini goat were 12.0 and 88.0 respectively. The results obtained from this study revealed the polymorphism pattern of the calpastatin gene in Tali and Raini goats.

Several methods including PCR-RFLP a good method to determine gene polymorphism is Calpastatin gene in domestic animals (Palmer *et al*. 1998).

Study on calpastatin gene in goat is very limited (Zhou and Hickford, 2008), this result shows that the polymorphism was detected in *CASTI* segment, as previously observed (Palmer *et al*. 1998) and (Casas *et al*. 2006).

Table 1 Observed alleles and genotypic frequencies for *CAST* gene in Tali and Raeini goats

Items	A	B	AA	BB	AB
Tali	0.47	0.53	38.4	9.6	52.0
Raeini	0.12	0.88	17.0	71.0	12.0

Table 2 Estimated statistically parameters for *CAST* gene in Tali and Raini gots

Items	Exp-Het	Exp-Hom	$Het_{(Nei)}$	Ave-Het	Obs-Hom	Obs-Het
Tali	0.533	0.467	0.51	0.51	0.48	0.52
Raeini	0.139	0.871	0.70	0.70	0.88	0.12

Small ruminants calpastatin gene mRNA transcript variants 2 and 4 have also been reported (Zhang *et al*. 2012). Who observed frequencies of 61, 36 and 3% of the MM, MN, and NN genotypes respectively in Iranian karakul sheep and Iranian kurdi sheep, the frequencies of 76, 24 and 0% were observed for MM, MN and NN genotype respectively, showing the existence of M allele more frequent then N allele (Nassiry *et al*. 2006).

Similar data were found by (Mohammadi *et al*. 2008). There are several studies on the association of *CAST* gene polymorphism with meat quality by PCR-RFLP analysis in animals (Schenkel *et al*. 2006). The polymorphism in the *CAST* in goat was also reported by other researchers using PCR-RFLP technique (Zhou and Hickford, 2008). Higher frequencies of *CAST* gene A allele compared to the B allele have been reported in Brangus (0.78) and Pardo Suico (0.80) cattle (Asadi and Khederzadeh, 2015). Reported a significant association between A allele of bovine *CAST* gene and meat tenderness (Kuryl *et al*. 2003).

Association between the D and F alleles of porcine *CAST* gene and meat quality traits was also reported (Kapelanski *et al*. 2004). The polymorphism in the exon 1 of the CAST in sheep was also reported by other researchers using PCR-RFLP technique (Mohammadi *et al*. 2008). In goats and bovine the exon 6 of *CAST* gene were investigated for polymorphisms and a number of allelic variants were identified in these species (Zhou and Hickford, 2008).

Two allelic systems of polymorphic variants (M and N) in the region of ovine *CAST* locus have been described by PCR-RFLP method (Shahroodi *et al*. 2005). The present study was the first attempt for identification of *CAST* gene variation in Tali and Raini goats. Further studies are required to investigate the relationship between *CAST* gene polymorphisms and performance traits in Tali and Raini goat.

CONCLUSION

The PCR-RFLP analysis of calpastatin gene in Tali and Raini goats revealed high level of polymorphism. This information could be utilized in future breeding plan to exploit the genetic potential of tali and raeni goats. The Tali and Raini goats breed showed genetic diversity for the calpastatin gene, but the polymorphism found in the *CAST* gene may be helpful in selection programs for genetic improvement of meat traits. Though, previous to submission in the genetic development of the indigenous goat breeds, the relationship of these polymorphisms with meat traits needs to be recognized.

REFERENCES

Alfnes F., Rickertsen K. and Ueland Ø. (2008). Experimental evidenceof risk aversion in consumer markets: the case of beef. *Appl. Econom*. **40,** 3039-3049.

Asadi N. and Khederzadeh S. (2015). Polymorphism of candi-date genes for meat quality in sheep. *Middle-East J. Sci. Res*. **23,** 2001-2004.

Averna M., Tullio R.D., Passalacqua M., Salamini F., Ponteremoli S. and Mellon E. (2001). Changes in intracellular calpastatin localization are mediated by reversible phosphorylation. *Biochem. J*. **354,** 25-30.

Bastos E., Cravador A., Azevedo J. and Guedes-Pinto H. (2001). Single strand conformation polymorphism (SSCP) detection in six genes in the portuguese indigenous sheep breed "Churra da Terra Quente". *Biotechnol. Agron. Soc. Environ*. **5,** 7-15.

Busch W.A., Stromer M.H., Goll D.E. and Suzuki A. (1972). Ca2+-specific removal of Z lines from rabbit skeletal muscle. *J. Cell. Biol*. **52(2),** 367-381.

Casas E., White S.N., Wheeler T.L., Shackelford S.D., Koohmaraie M., Riley D.G., Chase C.C.J., Johnson D.D. and Smith T.P. (2006). Effects of calpastatin and micro-calpain markers in beef cattle on tenderness traits. *J. Anim. Sci*. **84,** 520-525.

Goll D.E., Thompson V.F., Li H., Wei W. and Cong J. (2003). The calpain system. *Physiol. Rev*. **83,** 731-801.

Kapelanski W., Grajewska S., Kurył J., Bocian M., Jan-kowiak H. and Wisniewska J. (2004). Calpastatin (*CAST*) gene polymorphism and selected meat quality traits in pigs. *Anim. Sci*. **22,** 435-411.

Koohmaraie M., Kent M.P., Shackelford S.D., Veiseth E. and Wheeler T.L. (2002). Meat tenderness and muscle growth: is there any relationship? *Meat Sci*. **62,** 345-352.

Kuryl J., Kapelanski W., Pierzchała M., Grajewska S. and Bocian M. (2003). Preliminary observations on the effect of calpastatin gene (*CAST*) polymorphism on carcass traits in pigs. *Anim. Sci. Pap. Rep*. **21,** 87-95.

Mohammadi M., Beigi Nasiri M.T., Alami-Saeid K., Fayazi J., Mamoee M. and Sadr A.S. (2008). Polymor-phism of calpastatin gene in Arabic sheep using PCR-RFLP. *African J. Biotechnol*. **7,** 2682-2684.

Murachi T. (1983). Calpain and calpastatin. *Trends Biochem. Sci*.

8, 167-169.

Nassiry M.R., Tahmoorespour M., Javadmanesh A., Soltani M. and Foroutani Far S. (2006). Calpastatin polymorphism and its association with daily gain in Kurdi sheep. *Iranian J. Biotechnol*. **4,** 188-192.

Palmer B.R., Robert N., Hickford J.G.H. and Bickerstaffe G. (1998). Rapid Comunication: PCR-RFLP for *MSPI* and *NCOI* in the ovine calpastatin gene. *J. Anim. Sci*. **76,** 1499-1500.

Palmer B.R., Robert N. and Kent M.P. (1999). A candidate gene approach to animal quality traits. *Proc. Soc. Anim. Prod*. **57,** 294-296.

Rohrer G.A., Nonneman D.J., Miller R.K., Zerby H. and Moeller S.J. (2012). Association of single nucleotide polymorphism (SNP) markers in candidate genes and QTL regions with pork quality traits in commercial pigs. *Meat Sci*. **92,** 511-518.

Schenkel F.S., Miller S.P., Jiang Z., Mandell I.B., Ye X., Li H. and Wilton J.W. (2006). Association of a single nu-cleotide polymorphism in the calpastatin gene with carcass and meat quality traits of beef cattle. *J. Anim. Sci*. **84,** 291-299.

Shahroodi F.E., Nassiry M.R., Valizadeh R., Nosrati M., Javadmanesh A. and Tahmourespour M. (2005). The genetic polymorphism of calpastatin gene in Karakul sheep. *J. Agric. Sci. Natal. Res*. **2,** 1-10.

Yeh F.C., Yang R.C., Timothy B.J., Ye Z. and Judy M. (1997). Popgene, the User Friendly Shareware for Population Genetic Analysis. Molecular Biology and Biotechnology Center. Alberta, Cnada.

Zhang Z.R., Jiang X.S., Du H., Zhu Q., Li X.C., Yang C.W. and Liu Y.P. (2012). Characterization of the expression profiles of calpastatin (*CAST*) gene in chicken. *Mol. Biol. Rep*. **39,** 1839-1843.

Zhou H. and Hickford J.G. (2008). Allelic variation of the bovine calpastatin (*CAST*) gene. *Mol. Cell. Probes*. **22,** 129-130.