Proceedings

of the British Society of Animal Science

2007

Genetic variability in beta-lactoglobulin, calpastain and calpain loci in Kurdi sheep

Mohammad Reza Nassiry, Feridoun Eftekhari Shahroudi, Ali Javadmanesh
Excellence Center for Animal Science-Ferdowsi University of Mashad, Mashad, Iran
Email:m_nassiry@yahoo.com

Introduction DNA-based molecular methods have made possible genotyping of animals of any age and sex for milk and meat genes, thus providing a potentially more efficient and flexible selection tool. Among specific genes that may affect economically important traits in sheep, the Beta-lactoglobulin (BLG) locus has been extensively studied [Barillet et al., 2005]. The genotype BB of BLG seems to be associated with higher milk yield; on the other hand genotypes AA and AB seem to be superior in protein and casein content and crude yield [Garzon et al., 1992]. Another genes intensively investigated in farm animal are that of calpastatin (CAST) and calpain (CAPN). Calpastatin and calpain (CAPN) deserves special attention because of their major role in meat production. The calpain-calpastatin system (CCS) comprises a family of calcium dependent neutral proteases. The CAPNs have been shown to play the major role in post mortem tenderization in beef, lamb, and pork by degrading specific muscle structural proteins. The aim of the present study was to identify genotypes of BLG, CAST and CAPN genes in Kurdi sheep breed by PCR.

Materials and methods Blood samples were randomly collected from 100 pure bred Kurdi sheep from Kurdi breeding station located in Shirvan, Mashhad, Iran. DNA was extracted from 100 µl of blood by Guanidinium-Thiucianate Silica gel. Method. 1 µl DNA was amplified in a total volume of 20 µl PCR mix using the Biometra T Personal Ver: 1.11 thermocycler. Primers were designed by Primer Premier 5 software according to X12817 Gene Bank accession number for Beta-lactoglobulin, AY834765 for CAST and J05065 for CAPN. Products of amplification were recognized by electrophoresis on 1.5% agarose gel stained with etithium bromide. Five µl of PCR product was incubated for 5 h at 37 °C with 5 units of *Rsa*I and *Msp*I enzymes for BLG and CAST genes, respectively. Digestion products of were separated by electrophoresis on 8% non-denaturant polyacrylamid gel and visualized after silver staining. Polymorphisms of CAPN locus were detected using SSCP method on 8% non-denaturing polyacrylamid gel with 10 % glycerol. The frequencies of genotypes, alleles, mean expected, observed and Nei's heterozygosities and Hardy-Weinberg equilibrium test were calculated using PopGene32 (ver 1.31) program [http://cc.oulu.fi/~jaspi/popgen/popdown.htm].

Results Three loci were polymorphic in Iranian Kurdi sheep. The genotypes AA/AB/BB for the BLG, MM/MN for the CAST and AA/AB for the CAPN locus were observed. Table 1 shows the allele frequencies for BLG, CAST and CAPN genes in the Iranian Kurdi sheep

Table 1: Allelic and genotype frequencies, observed heterozigosity, expected heterozigosity, average heterozigosity and Nei values for BLG, CAPN and CAST loci.

Locus	A	B	AA	AB	BB	Obs_Het	Exp_Het*	Nei**	Ave_Het	χ^2
BLG	0.51	0.49	24%	54%	22%	0.5400	0.5023	0.4998	0.4998	0.5686
CAPN	0.96	0.04	92%	8%	0%	0.0824	0.0794	0.0790	0.0790	0.1336
CAST***	0.88	0.12	76%	24%	0%	0.2400	0.2123	0.2112	0.2112	1.7743

* Expected heterozygosity were computed using Levene (1949)
** Nei's (1979) expected heterozygosity
***Alleles for CAST locus have shown in text with M and N but in table M=A and N=B.

Conclusions Our results showed that PCR-RFLP and PCR-SSCP were appropriate tools for evaluating genetic variability. This study was the first using polymorphism of BLG, CAST and CAPN loci to understand genetic variability of Kurdi sheep in Iran. The present study may be regarded as the beginning of attempts to understand the genetic variability of native sheep breed in Khorasan state and identification of association between genotypic variants and productive parameters for future study.

Acknowledgements This investigation was supported by Excellence Center for Animal Science, Agriculture Faculty, Ferdowsi University of Mashhad.

References

Barillet F., Arranaz J., Carta A. Mapping quantitative trait loci for milk production and genetic polymorphisms of milk proteins in dairy sheep// Genet. Sel. Evol. 2005, vol. 37, pp: 109-123.
Boom R., Sol C.J., Salimans M.M. et al. Rapid and simple method for purification of nucleic acids// J. Clin. Microbiol. 1990, vol. 28(3), pp: 495-503.
Garzon A.I. and Martinez J. β-Lactoglobulin in Manchega sheep breed: Relationship with milk technological index in handcraft manufacture of manchego cheesse// Anim. Genet. 1992, vol. 23, suppl.1. p :106.