

## STUDY ON THE CALPAIN GENE POLYMORPHISM BY PCR-SSCP AND ITS RELATION TO AVERAGE DAILY GAIN IN IRANIAN BALUCHI SHEEP

**Mojtaba tahmoores pour, fereydoun eftekhari shahrudi, reza valizadeh, mohammad reza nassiry, ali reza heravi moussavi, ali javadmanesh**

**Department of animal science, faculty of agriculture, ferdowsi university, mashhad, iran. 91775-1163.**

### ABSTRACT

Different genotypes for calpain (CAPN) were determined by PCR-SSCP (single strand conformation polymorphism) method in Baluchi sheep. Blood samples were taken from 202 pure bred Baluchi sheep, in Abasabad Breeding Station near to Mashhad, the capital of Khorasan province, Iran. CAPN genotypes were analyzed by SSCP method following DNA extraction and PCR reaction. Three CAPN genotypes AA, AB and BB with frequencies of 0.30, 0.53 and 0.17, were detected respectively. The  $\chi^2$  test confirmed that the Hardy-Weinberg equilibrium was existed within the population. Relationships between data and genotypes were investigated using mixed models. The average daily gain (ADG) from birth to three months of age was affected by birth type, birth weight (BW) and the square of BW. Different genotypes had similar trend in average daily gain performance. It was concluded that CAPN polymorphism may not be a single suitable marker in selection programs for improving the average daily gain in Baluchi sheep alone.

Key Words: CAPN, Polymorphism, PCR-SSCP, Baluchi sheep, daily gain.

### INTRODUCTION

Several DNA polymorphisms have been considered as potential tools for selection programs. DNA-based molecular methods have made genotyping of animals of any age and sex for milk and meat genes possible, thus providing a potentially more efficient and flexible selection tool. Selection efficiency, however, depends on allelic frequencies in the breeds and on the effect of these polymorphisms on dairy and meat traits and technological properties of milk and meat. It has been suggested that CAPN-mediate proteolysis of myofibril protein is the main reason for meat tenderness during postmortem storage of carcasses (Kocwin et al. 2003). The CAPN-calpastatin system (CCS) comprises a family of calcium dependent neutral proteases. The CAPN and calpastatin are specific inhibitors of the CAPN which regulates their *in-vivo* activity. The CCS is found in most animal tissues and influences many important processes including muscle development and degradation, meat tenderization post-mortem, cataract formation and fertility (Choi et al., 2002). 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 level of postmortem calpastatin appears to be critical in determining the ultimate tenderness of aging muscles (Koohmaraie, 1992). Increased rate of skeletal muscle growth can result from a decreased rate of muscle protein degradation and this is associated with a decrease in activity of the CAPN system, due principally to a large increase in calpastatin activity. It is now accepted that CAPN-mediated degradation of myofibrillar proteins is responsible for the post mortem meat tenderization, which occurs during storage at refrigeration temperatures. Two CAPN alleles (A and B) from exons 5 and 6 have been identified and are easily detected by PCR amplification and SSCP process (Chung et al., 1999).

The aim of the present study was to identify genotypes of CAPN genes by PCR and their relationship with ADG in Iranian Baluchi sheep.

### MATERIALS AND METHODS

#### DNA extraction and PCR

Blood samples were randomly collected from 202 pure bred Baluchi sheep from Abasabad Breeding Station near to Mashhad. DNA was extracted from 100 micro litters as described by Boom et al. (1989).

Thirty to fifty ng of DNA was amplified in a total volume of 25  $\mu$ l PCR mixture using the Biometra T Personal Ver: 1.11 thermocycler. The PCR mixture contained: 2.5  $\mu$ l PCR buffer 10-X, 2.5 mM MgCl<sub>2</sub>, 200  $\mu$ M dNTPs, and 3  $\mu$ l mixture of oligonucleotids (10 pm from each primer) and 1 U *Taq* DNA polymerase.

PCR program included a preliminary denaturizing at 95°C, followed by 35 cycles, denaturing at 94°C for 45 sec annealing at 59°C for 1 min, extension at 72°C for 1.5 min and 10 min at 72°C as final extension. The ovine m-CAPN regulatory gene (CAPN456) was amplified with primers:

CAPN456F: 5'-AACATTCTCAACAAAGTGGTG-3' CAPN456R: 5'-ACATCCATTACAGCCACCAT-3' (Gene bank accession no. J05065). Products of amplification were recognized by electrophoresis on 1.5% agarose gel stained with ethidium bromide.

#### SSCP

For the genotyping of CAPN locus, PCR products were diluted with 12  $\mu$ l of running buffer. Running buffer included: 800ul formamid, 100ul bromophenol blue 1%, 100ul xylenecyanol 1%, 2ul 0.5 M EDTA and 1ul 10M NaOH. After heating at 95°C for five minutes, they were immediately placed on ice. Polymorphisms were detected using 8% non-denaturing polyacrylamid gel with 10 % glycerol. The mixture was electrophoresed for 3-4 hours at 250 V and 10°C. DNA fragments were visualized using silver staining method.

### Statistical analysis

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. The relationship between data and genotypes were analyzed by JMP program (4.0.4) using the following mixed model:

$$Y_{ijkln} = \mu + P_i + Q_j + R_k + S_l + I_{jl} + b_1(X_{ijkln} - \bar{X}) + b_2(X_{ijkln}^2 - \bar{X}^2) + e_{ijkln}$$

Where,  $Y_{ijkln}$  = average daily gain on each animal,  $\mu$  = overall mean for each trait,  $P_i$ ,  $Q_j$ ,  $R_k$ ,  $S_l$  = fixed effects of dam, birth type, genotype, sex and interaction of sex and birth type respectively.  $b_1(X_{ijkln} - \bar{X})$ ,  $b_2(X_{ijkln}^2 - \bar{X}^2)$  and  $e_{ijkln}$  = was the random effect of BW, square of BW and error respectively. Interactions effects were non-significant and discarded for final analysis. Weaning weight added to model for account of ADG from 3 to 6 months.

### RESULTS

The ovine CAPN II regulatory gene, exon 5 and 6 including intron (CAPN456), was amplified. SSCP method differentiated 3 conformations: AA, AB and BB with frequencies of 0.303, 0.523 and 0.174, respectively. Two alleles (A and B) were observed with frequencies of 0.564 and 0.435. Table 1 shows the allele frequency for CAPN genes in Baluchi sheep.

The heterozygous genotype AB was most frequent genotype in Baluchi breed which was detected at 52.5% of the studied individuals (Table 1). Homozygous genotypes of AA and BB were observed at frequencies of 30.2% and 17.3%, respectively. Mean observed heterozygosity (0.524) was slightly higher than the expected mean heterozygosity (0.492); Nei's heterozygosity value (0.491) was similar to the mean expected heterozygosity (Nei 1979). No deviation from Hardy-Weinberg equilibrium was detected.

Relationship between data and genotypes were investigated using the mixed model. The average daily gain from birth to three months of age was not affected by genotype, dam, sex and interaction between birth type and sex but it was affected by birth type, BW and square of BW significantly ( $p < 0.05$ ) (Table 2).

### DISCUSSION

Certain proteolytic enzymes and their inhibitor genes, encoding proteins of a known function and playing an important role in physiological processes, are described as candidate genes. Their polymorphism was examined for their effect on corresponding production traits (Juszczuk-Kubiak et al. 2002). The CAPN gene was investigated as a potential candidate gene for quantitative trait locus (QTL) affecting meat tenderness (Koochmaraie 1992). Observed heterozygosity (0.522), expected heterozygosity (0.492), Nei's heterozygosity (0.491) and average heterozygosity (0.491) of CAPN locus for Baluchi sheep were relatively similar. Chung et al. (1999) analyzed the same exon with the same methodology and primers and found 0.69 and 0.31 frequencies for A and B alleles, respectively. Mean observed heterozygosity (0.522) and Nei's heterozygosity in Baluchi sheep (0.491) and in Karakul (Eftekhari Shahroudi et al. 2005) sheep (0.25) were higher.

Association of genetic variants of the ovine CAPN gene with growth and meat tenderness examined in many researches. It may be beneficial to use CAPN genotypes in marker assisted selection programs to improve growth traits of lambs (Chung et al., 2000). Davis et al (2000) found difference in CAPN4 genotype for weaning weight of Angus bulls. Therefore CAPN regulatory subunit and CAPN may take part for variation in meat tenderness. Our data showed that PCR-SSCP is appropriate tool for evaluating genetic variability. SSCP method proved to be an effective technique for the detection of genetic polymorphism. Since the different genotypes had similar trend in average daily gain performance, it was concluded that CAPN polymorphism might not be a suitable single marker in selection programs for improving average daily gain in Baluchi breed of sheep.

### REFERENCES

- Boom, R., C. J. A. Sol, and M. M. M. Salimans. 1990. Rapid and simple method for purification of nucleic acids. *J Clinical Microbiology*. 28(3): 495-503.
- Choi, B. H., B. J. Ahn, K. Kook, S. S. Sun, K. H. Myung, S. J. Moon, K. H. Kim and J. H. Kim. 2002. Effects of dietary treatment, gender, and implantation on Calpain/Calpastatin activity and meat tenderness in skeletal muscle of Korean native cattle. *Asian-Aust. J. Anim. Sci.* 15 (11): 1653-1658.

- Chung, H. Y., M. E. Davis, H. C. Hines and D. M. Wulf. 1999. Effect of the CAPN proteolysis and CAPN genotype on meat tenderness of Angus Bulls, *J. Anim. Sci.*, vol. 77, Supplement 1, pp: 31.
- Chung, H. Y., M. Davis and H. Hine. 2000. Relationship of genetic variants in the ovine CAPN regulatory gene with growth. *J. Anim. Sci.* vol. 28. supplement2, pp: 55.
- Davis, M. E., H. Y. Chung, and H. C. Hines. 2000. Effect of the CAPN system on the growth of Angus bulls. *J. Anim. Sci.* 30: 1050-1058.
- Eftekhari Shahroudi, F., M. R. Nassiry, R. Valizadeh, A. Javadmanesh and M. Tahmoresur. 2005. The genetic polymorphism of calpastatin gene in karakul sheep, *J. Agricultural Science and Natural Resources of Khazar.* 2(3): 1-10.
- Juszczuk-Kubiak, E., S. Jozef Rosochacki and K. Wicinska. 2002. A note on restriction fragment length polymorphism for *HhaI* in the bovine CAPN gene. *Anim. Sci. Paper and Reports*, Vol. 20(3): 181-185.
- Kocwin, M. P. and J. Kuryl. 2003. The effect of interaction between genotype at loci CAST, RYRI and RN on pig carcass quality and pork traits. *Ainm. Sci. paper and report*, vol. 21(1): 61-65.
- Koohmaraie, M. 1992. The role of the Ca<sup>++</sup> dependent protease (CAPNs) in postmortom proteolysis and meat tenderness. *Biochimie*, vol. 74: 239-245.
- Nei, M. and W. H. Li. 1979. Mathematical model for studying genetic variation in terms of the restriction endonucleases. *Proceeding of the National Academy of Science. USA.* 76: 5269-5273.

**Table 1.** Allele frequencies, observed heterozygosity, expected heterozygosity, average heterozygosity, Nei and  $\chi^2$  values for CAPN loci.

Locus	Allele A	Allele B	Obs_Het	Exp_Het*	Nei**	Ave_Het	$\chi^2$
0.492	0.491	0.491	0.84	0.564	0.435		0.524

\* Expected heterozygosity were computed using Levene (1949)

\*\* Nei's (1973) expected heterozygosity

**Table 2.** Analysis of variance.

M.NO	177	177	159028.31	1.47	0.18
B.Type	1	1	5033.57	8.22	0.01
G2	2	2	1274.03	1.04	0.37
Sex	1	1	233.99	0.38	0.54
B.Type*Sex	1	1	550.09	0.90	0.36
BW	1	1	8238.99	13.45	0.00
BW*BW	1	1	7750.11	12.65	0.00

**Table 3.** Mean and standard error of ADG from 0-3 months for calpain genotypes (gr).

Genotypes	AA	AB	BB
0-3 month daily gain	184.6 ± 13.3	188.6 ± 10	172.6 ± 21.1