International Research Journal of Engineering and Technology (IRJET) e-ISSN: 2395-0056
Volume: 07 Issue: 10 | Oct 2020 www.irjet.net p-ISSN: 2395-0072

Molecular Characterization of CAST Gene Polymorphism in Iraqi Awassi Sheep

Mervan Bayraktar

College of Agriculture, Animal Science, Selcuk University, Konya, Turkey

---

ABSTRACT: The aim of this study is to investigate CAST gene polymorphism in the males of Iraqi Awassi sheep. PCR-RFLP method was used to detected CAST gene polymorphism. Three genotypes (MM, MN and NN) were detected at CAST (Exon 1C/1D) locus. The allele frequency of M and N was 0.60 and 0.40, respectively, while genotype frequency of MM, MN and NN were 0.47, 0.27 and 0.26 respectively. The chi-square χ^2 test showed an agreement to Hardy-Weinberg equilibrium (P>0.05).

Keywords: Awassi, CAST, polymorphism, PCR-RFLP, SNP.

1. Introduction

In every country in the world, over hundreds of years, livestock breeds have been improved to suit the conditions of the region and the environment. This improvement was achieved through natural selection, i.e. the death of weak animals that are unable to adapt to the circumstances, or by selective breeding and systematic selection, which aims to select individuals with distinct characteristics that are compatible with the conditions of the place and the needs of the human. One of the native breeds spread throughout Iraq Awassi sheep are strong, withstand harsh weather conditions, have good carcass quality and a low in fat (Al-Salihi et al. 2017; Al Qasimi et al. 2019). The ovine CAST gene plays an important role in muscle development and meat tenderness after slaughter and is a gene located on the chromosome 5, contains 32 exons 32, 2.701 bp transcript length and 786 residues of translation length (No: ENSOART00000019281.1) (Gabor et al. 2009; Balcıoğlu et al. 2014). The aim of this study is to determine CAST gene polymorphism in Iraqi Awassi sheep.

2. Material and method

A total of 95 (male) from Iraqi Awassi sheep were used in this study. Disodium EDTA containing tubes were used to prevent coagulation of blood during the collection of samples. Then, blood samples storage was carried out at -20°C until DNA extraction procedures. Blood samples were taken from the Tail Vein of animals. Genomic DNA was extracted from whole blood by using the phenol chloroform methods. 622 bp length of the CAST gene (Exon 1C/1D) region was amplified with forward (5'TGGGGCCCAATGACGCCATCGATG-3') and reverse (5'GGTGGAGCAGCACTTCTGATCACC-3') primers reported by (Palmer et al. 1998). The PCR was done in a reaction volume of 10 µL according to some modifications. The reaction consists of 5µL of 2X Dream Taq Green PCR Master Mix (Thermo Scientific), 0.30µL primer each primer forward and reverse (10 pmol) and 3.4µL ddH_2O which finally added to 1 µL genomic DNA. The cycling protocol followed with an initial denaturation at 95˚C for 3 min followed by 30 cycles of denaturation at 95˚C for 30 sec, annealing at 63˚C for 50 sec, extension at 72˚C for 60 sec with a final extension at 72˚C for 10 min. The PCR product of each sample (5 µL) and 1000 bp DNA ladder (Vivantis) was loaded in 2% (w/v) agarose gels in 0.5X Tris-Boric-EDTA (TBE) buffer and staining using ethidium bromide. The electrophoresis was carried out for 50 min at 100 V. The electrophoresis gel was examined on a UV trans-illuminator and bands were visualized and photographed. The PCR products of CAST gene were digested by *MspI* fast digest (Thermo Scientific). The reaction volume was 15 µL consisted of 5 µL PCR product, 8.5 µL ddH_2O, 1 µL 10X buffer and 0.5 µL restriction enzyme. The polymorphism of the cleaved fragments recognition was carried out by %2 agarose gel electrophoresis then the digested PCR products were obviously envisioned under UV light and scored in a gel documentation system.

3. Result and discussion

622 bp of PCR product was amplified. The PCR product was digested with *MspI* restriction enzyme. After digesting the PCR product by *MspI*, three genotypes (MM, MN and NN) were obtained. MM genotype was 336 bp and 286 bp; MN genotype was 622 bp, 336 bp and 286 bp; NN genotype was 622 bp (Figure 1). chi-square χ^2 test showed agreement to Hardy-Weinberg equilibrium (p>0.05). The allele and genotype frequency was 0.60(M) and 0.40(N); 0.47(MM), 0.27(MN) and 0.26(NN). The A allele frequency has been determined as 0.78 in the Kurdi sheep (Nassiry et al. 2006) and in the Zandi sheep (Khederzadeh et al. 2016), while the M allele frequency has determined as 0.77 in Awassi sheep (Jawasreh et al. 2017) and as 0.79 in Karakul sheep (Eftekhari et al. 2006).

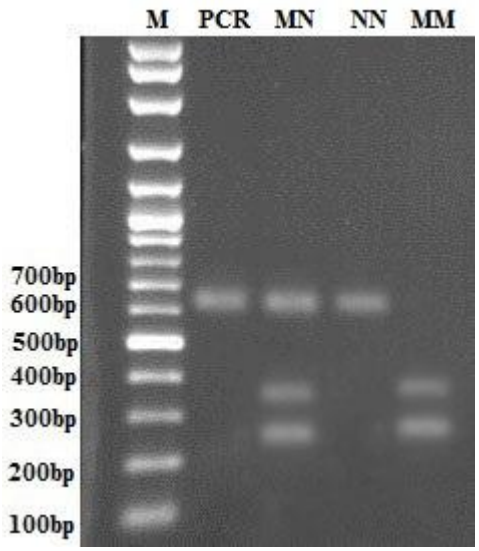

Figure 1. *PCR-RFLP analysis of the CAST/MspI polymorphism, 622 bp PCR fragment; 622 bp, 336 bp and 286 bp for MN genotype; 622 bp for NN genotype; 336 bp and 286 bp for MM genotype*

4. Conclusion

The genes show polymorphism in Awassi sheep so they can be considered important genetic markers, used as markers in genetic improvement programs for improving the growth traits of sheep breeds.

5. References

1. Al-Salihi, A., Al-Saadi, B., and Al-Anbari, N. 2017. Genotypes Relationship of Growth Hormone Gene Polymorphism with Some Productive and Reproductive Trait in Awassi Sheep. Journal of Biotechnology Research Center **11**: 26-33.

2. Al Qasimi, R.H., Hassan, A.F., and Khudair, B.Y. 2019. Effect of IGF-1 and GH Genes polymorphism on weights and body measurements of Awassi lambs in different ages. Basrah Journal of Agricultural Sciences **32**: 39-46.

3. Balcıoğlu, M., Karslı, T., Şahİn, E., Ulutaşc, Z., and Aksoy, Y. 2014. Determination of calpastatin (CAST) gene polimorphism in some native sheep breeds reared in Turkey by PCR-RFLP method. Tarim Bilimleri Dergisi **20**: 427-433.

4. Eftekhari, S.F., Nassiry, M.R., Valizadh, R., Heravi Moussavi, A., Tahmoores Pour, M., and Ghiasi, H. 2006. Genetic polymorphism at MTNR1A, CAST and CAPN loci in Iranian Karakul sheep. Iranian Journal of Biotechnology **4**: 117-122.

5. Gabor, M., Trakovická, A., and Miluchová, M. 2009. Analysis of polymorphism of CAST gene and CLPG gene in sheep by PCR-RFLP method. Scientific Papers Animal Science and Biotechnologies **42**: 470-476.

6. Jawasreh, K.I., Jadallah, R., Al-Amareen, A., Abdullah, A.Y., Al-Qaisi, A., Alrawashdeh, I.M., Al-Zghoul, M., Ahamed, M.K.A., and Obeidat, B. 2017. Association between MspI calpastatin gene polymorphisms, growth performance, and meat characteristics of Awassi sheep. Indian J. Anim. Sci **87**: 635-639.

7. Khederzadeh, S., Iranmanesh, M., and Motamedi-Mojdehi, R. 2016. Genetic diversity of myostatin and calpastatin genes in Zandi sheep. Journal of Livestock Science and Technologies **4**: 45-52.

8. Nassiry, M.R., Tahmoures, P.M., Javad, M.A., Soltani, M., and Foroutani, F.S. 2006. Calpastatin polymorphism and its association with daily gain in Kurdi sheep. **4**: 188-192.

9. Palmer, B., Roberts, N., Hickford, J., and Bickerstaffe, R. 1998. Rapid communication: PCR-RFLP for MspI and NcoI in the ovine calpastatin gene. J. Anim. Sci **76**: 1499-1500.