Research Article

Analysis of Polymorphism of Caplstatin and Callipyge Genes in Saudi Sheep Breeds Using PCR-RFLP Technique

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ABSTRACT

With the recent advances in molecular biology techniques, a great interest focused towards the improving livestock production in general and meat production in specific. In the present study is to identify the genetic polymorphism of calpastatin and callipyge genes (CAST and Clpy) related to meat production in two Saudi sheep breeds (Najdi and Harri). The detection was done using the restriction fragment length polymorphism for the polymerase chain reaction products (PCR-RFLPs). Genomic DNA was isolated from 20 animals from each breed; The PCR products were digested with *Msp*I restriction enzyme for calpastatin gene and *Faq*I restriction enzyme for callipyge gene. The results indicated the presence of polymorphism in the callipyge gene was detected homozygous genotype AA – 1.00 only in the two breeds understudy. The heterozygous genotype AG and homozygous genotype GG was not detected. Calpastatin locus digested with *MspI* had two genotypes MM and MN. The highest allelic frequency was for allele M allele but homozygous genotype NN has not been observed. The CAST locus had two genotypes MM (0.64 and 0.26 from Najdi and Harri) and MN (0.36 and 0.74 from Najdi and Harri), but homozygous genotype NN has not been observed. It may be concluded that CLPG locus is a monomorphic for population sheep kept in Saudi. The locus of CAST gene is polymorphic for breeds of sheep involved Najdi and Harri. Najdi sheep breed showed the highest observed heterozygosity for *CAST* gene.

Keywords: Sheep, PCR-RFLP, Calpastatin gene, Callipyge gene.

INTRODUCTION

At the present time, the consumers demand for sheep meat is not focused on quantitative traits only, but also qualitative traits of meat too. Animal breeders could accelerate the rate of genetic improvement attained in carcass composition and meat quality traits through the application of gene-assisted selection (GAS), based simply on incorporating some candidate genes in traditional breeding programs[1].

The Najdi or Nejdi is a breed of domestic sheep native to the Najd region of the Arabian Peninsula. Though it is primarily raised in Saudi Arabia, Nadji sheep are also present in Kuwait, Jordan, Oman, and Iraq[2]. The Najdi has a distinctive appearance that has even been celebrated in Saudi "sheep beauty pageants" not unlike livestock shows and sales in the West. They are a very tall breed, averaging 76-86 centimeters (30-34 inches) in height at the withers[2]. They have long, Roman nosed faces with drooping ears. Ewes are polled and rams may be either polled or have scars.

They are generally black with white faces and white on the legs and tail. Top Najdi ewes can sell for 20,000-30,000 Saudi riyals ($5,300-$8,000 USD), while rams which can sire many more offspring can fetch hundreds of thousands[2].

Najdi are highly adapted to life in desert conditions, though it is less drought tolerant than some breeds, such as the Awassi[3,4]. Though its meat may be consumed locally, it is especially valued for its milk and long, straight wool.

There are many of published articles on two genes associated with meat-related traits in varied sheep breeds, CAST and Callipyge genes[5].

Calpastatin (CAST) gene is located on the fifth chromosome of sheep and plays important roles in formation of muscles, degradation and meat tenderness after slaughtering[6]. It was also proved that there is a relationship between polymorphism in the calpastatin gene in sheep and slaughter traits such as lamb's body weight at birth and its growth rate until weaning[7]. Associations between variation in CAST and carcass and meat quality traits in sheep, there was also a genetic variation in the CAST gene [8-11]

The Callipyge (CLPG) gene is most documented gene for double muscle in sheep which causes a postnatal muscle hypertrophy that is localized to the pelvic limbs and loin[12]. The mutation cognizant of unique muscling phenotype in sheep was first discovered in 1983 in Oklahoma in Dorset breed. The locus of CLPG gene was mapped to the telomeric region of ovine chromosome 18[13]. The CLPG gene has unique inheritance pattern, which has been termed as polar overdominance[14]. The term polar overdominance was used to describe the genetic model in which only heterozygous individuals that were receiving the newly identified allele from their sire expressed the unique phenotype[14]. Callipygephenotypic effects were previously estimated in heterozygous lamb but the mutant CLPG allele have to be inherited from sire and normal allele have to be inherited from dam. The others combination of allele can be classified as non carrier lambs with normal phenotype[15].

The aim of the present study is to identify the genetic polymorphism for two genes correlated (CAST and Clpy) to meat production in two Saudi sheep breeds (Najdi and Harri), the identification was done using the Polymerase Chain Reaction-Restriction Fragment Length polymorphisms methodology (PCR-RFLPs).

MATERIALS AND METHODS

Animals

A total of 40 animals represented the two Saudi sheep breeds understudy, Najdi and Harri; were randomly sampled (20 sample per breed). The Saudi sheep breeds (20 Najdi and 20Harri) obtained from the slaughter house in Jeddah, Faculty of Science, King Abdel-Aziz University. Blood samples were collected in tubes containing EDTA as anticoagulant and transported to the laboratory under cooled conditions.

DNA Extraction

DNA was extracted and purified from blood samples using the whole blood salting out technique described by Miller.[16] DNA concentration and purity were determined using a UV spectrophotometer at optical density of 260 and 280 nm.

Polymerase Chain Reaction (PCR)

Polymerase chain reactions (PCR) were performed using specific primers for each gene under study. Details of the primer sequences and the optimized PCR conditions are listed in Table 1, amplification reaction was carried out in a 25 ul volume containing 100 ng genomic DNA, forward and reverse primer (both at concentration 10 pmol/µl), 1U *Taq* polymerase, 2.5 µl Taq polymerase buffer, four dNTPs (each at final concentration of 2.5 mM/µl) and H_2O up to a total volume of 25 µl. The general PCR program for the amplification of the genes included in the current study was: initial denaturation: 95 °C for 3 min., 95 °C for15 sec. (denaturation), 58–63 °C for 30–60 sec. (annealing depending on the gene) and 72 °C for 30 sec. up to 35 cycles, then final extension at 72 °C for 5 min. and finally storage at 15 °C forever.

For optimization the PCR the temperature and the time of the annealing temperature were changed. The success of PCR was tested after running some of the products on 2% horizontal agarose electrophoresis gel, and staining with ethidium bromide.

Restriction Fragment Length Polymorphism (RFLP)

Ten microliters from the PCR products were digested with 5 units of the fast restriction enzyme including specific buffer (Fermentas, Germany) specific for each gene (Table 1) in a final reaction volume of 15 µl. The reaction mixture was incubated at 37 °C in water bath for 30 minutes.

After restriction digestion, the restricted fragments were identified after running in horizontal gel electrophoresis system (2-3 % agarose) and staining with ethidium bromide. The 100-bp ladder was used as molecular size marker. The bands were visualized under UV light and the gels were photographed using digital gel documentation system (Bio-Rad, USA). The allele sizes were determined using free software named Lab. Image V2.7. It is dispersed free from Proland company (Germany), from the internet through the web page:

http://www.labimag-ing.com/servlet/engine/home/start.html.

Table 1: The Identification of the Primers and Restriction Enzymes.

Gene	The primer sequences	Size	Annealing temperature	Restriction Enzyme	Reference
Calpastatin	TGGGGCCCAATGACGCCATCGATG GGTGGAGCAGCACTTCTGATCACC	622 bp	59 °C	MSP1	Suleman[17]
Callipyge	TGA AAA CGT GAA CCC AGA AGC GTC CTA AAT AGG TCC TCT CG	426 bp	58 °C	*FaqI*	Gabor[11]

Statistical Analysis

The allelic frequencies were calculated using Popgene software package version 3.2[18].

RESULTS AND DISCUSSION

Calpastatin

The amplified CAST resulted in a DNA fragment with 622 bp including the sequences of exon and intron regions from a portion of the first repetitive domain by PCR technique is shown in Figure 1.

The digestion of 622 bp PCR product of CAST gene with restriction end nuclease enzyme (*MspI)* differentiated alleles N and M. The *MspI* digestion of the PCR products produced fragments of 336 bp and 286 bp for allele M, but the allele N was not digested (Figure 2).

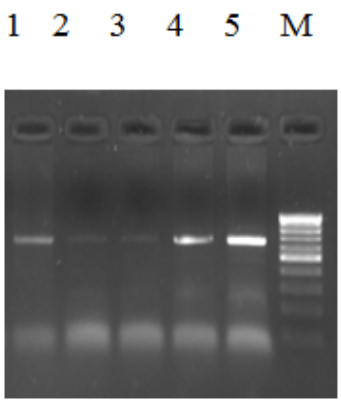

Figure 1: Ethidium bromide-stained gel of PCR products representing amplification of CAST gene in Saudi sheep breeds. Lane M: 50bp ladder marker, Lanes 1-5: 622bp PCR products amplified from sheep DNA

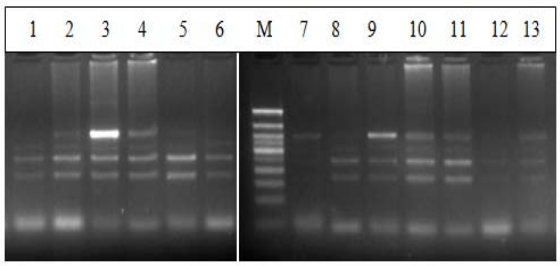

Figure 2: DNA electrophoric pattern of *CAST* amplicons after digestion with *MSP1* end nucleases, Lane M, 100pb DNA ladder, lane 2, 3, 4, 7, 9, 10, 11, 13: genotype NM (602 , 336 and 286pb) and lane 1, 5, 6, 8, 12: genotype MM (336 and 286).

The homozygous genotype NN (622 bp) was not observed in the two sheep breeds studied. The heterozygous genotype NM (622 bp, 336 bp and 286 bp) and the Homozygous genotype MM (336 bp and 286 bp), both were detected in two breeds involved.

These results are in agreement with the polymorphism detected in CAST gene previously observed by Palmer[19] in Dorset sheep, Chung[20] in mixed population of 96 sheep of Tsigai, Improved Valachian, East Friesian, Lacaune and Lacaune x Tsigai breed and Gabor[11] in local sheep raised in Slovakia and found only two genotypes MM and NM. On the other hand, Sumantri[17] found only NN and NM genotypes in Indonesian Jonggol sheep. Results of allele frequencies and genotypes of CAST gene in the two breeds studied are shown in Table 2. The genotype frequencies for MM were: 0.64 and 0.26 from Najdi and Harri, while NM genotype was 0.36 and 0.74 after digestion with *Msp1* (Table 2).

Frequencies of allele M were 0.66 and 0.87 in Najdi and Harri breeds in Saudi, respectively, while 0.34 and 0.13 for allele N in Najdi and Harri breeds in Saudi (Table 2).

Table 2: Genotypic, allelic frequencies and χ2 estimates of CAST gene digested with *Msp*1.

Breeds	Genotypic Frequency			Allelic Frequency	
	(NN)	(NM)	(MM)	N	M
Najdi	0	0.36	0.64	0.34	0.66
Harri	0	0.74	0.26	0.13	0.87

The value of the observed heterozygosity was (0.64, 0.26) and the value of expected hetrozygosity was (0.42, 0.20) for Najdi and Harri respectively (Table 3).

The *CAST* locus *MspI* had χ2 value of 3.95, 0.512 f or Najdi and Harri breeds respectively (Table 3).

Polymorphism of CAST gene has also been reported in a variety of other sheep in the world such as the Dorset sheep[19], Kurdi Iranian sheep breed[21]; Karakul Iranian sheep breed[22], Merino, Corriedale, Romney, Poll Dorset, and crossbred NZ sheep in New Zealand[9], Tsigai sheep, Valachian sheep, East Friesian sheep, Lacaune sheep, and Lacaune Tsigai crossbred sheep[11].

There was a tendency of these two sheep breeds to have higher frequencies of M allele than the N allele (Table 2). Other studies also showed similar high frequency of allele M, and low frequency of allele N like our results. Shahroudi[22] reported that the allelic frequencies of gene CAST locus *MspI* in Karakul sheep frequencies for M allele were 0.79 while N was 0.21. Gabor[11] also reported that the genotyping results of CAST gene locus *MspI* in Tsigai sheep, Improved Valachian, Lacaune, East Friesian, and Tsigai sheep × Lacaune sheep had allele frequencies of M - 0.94 and N - 0.06.

In contrast, the result of the current study differs from that reported by Sumantri[10] who found that the allele N frequency was higher than allele M in Indonesian Jonggol sheep breed.

Table 3: Observed heterozygosity (Ho) and expected heterozygosity (He) of alleles and χ2 estimates of *CAST* gene digested with *Msp1.*

Breeds	Observed Het.	Expected Het.	χ2
Najdi	0.64	0.42	3.95
Harri	0.26	0.20	0.512

Callipyge Gene

In our study, the genotyping of *CIPG* gene were done using PCR-RFLPs methodology. A 426bp fragment of *CIPG* locus was amplified, then this fragment was digested with the restriction enzyme *FaqI*, the digested products were characterized after running on horizontal agarose gel electrophoresis.

The *Faq*I digestion of the PCR products produced digestion fragments of 395 bp and 31 bp for allele G and 278 bp, 117 bp and 31 bp for allele A. The total population of sheep was monomorphic for CLPG gene, because we detected allele A only (Fig. 3 & 4). The mutant allele G was not detected in all breeds and involved in this study.

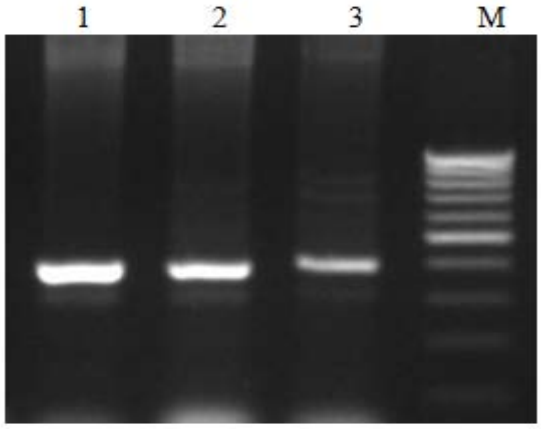

Figure 3: Ethidium bromide-stained gel of PCR products representing amplification of CLPG gene in Saudi sheep breeds. Lane M. 50-bp ladder marker, Lanes 1-3: 426bp PCR products amplified from sheep DNA

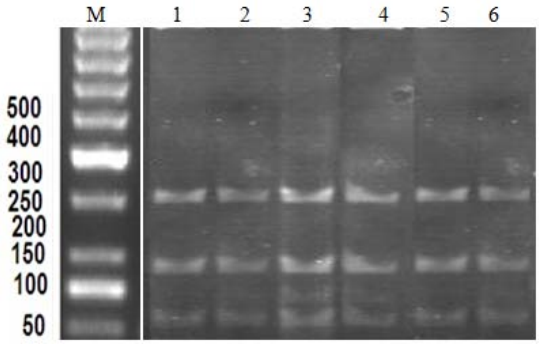

Figure 4: Representatively results of analysis PCR-RFLP for CLPG gene by restriction enzyme *FaqI* on 3 % agarose gel. Lane M: ladder 100 bp (Fermentas), Lanes 1-6 : AA - genotype (278 bp, 117 bp, 31 bp) in Saudi sheep breeds

These results are in agreement with the monomorphic detected in ClPG gene previously observed by Gabor[11] in local sheep raised in Slovakia and detected homozygous genotype AA – 1.00 only. The heterozygous genotype AG and homozygous genotype GG was not detected. Qanbari[23] did not detected CLPG mutation in experimental flock of Afshari breed. Jackson[24] detected equal results of frequence for genotype AA in 88 rams kept in Slovakia. The occurrence the mutant allele G was determinate for breeds of sheep than Dorset, Rambouillet and Hampshire, respectively**.**

CONCLUSION

It may be concluded that CLPG locus is a monomorphic for population sheep kept in Saudi. It was detected the genotype AA with frequency 1.00.

The locus of CAST gene is polymorphic for population of sheep involved Najdi and Harri. Najdi sheep breed showed the highest observed heterozygosity for *CAST* gene.

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REFERENCES

1. Anderson L, Genetic dissection of phenotypic diversity in farm animals. Nat. Rev. Genet. 2, 2001, 130–138.
2. FAO, “Breeders hold first pageant for Saudi-bred sheep”. MSNBC (Associated Press). Oct. 31, 2008.
3. Alamer M, Adel A, Effect of water deprivation and season on feed intake, body weight and thermoregulation in Awassi and Najdi sheep breeds in Saudi Arabia. Journal of arid environments, 59, 2004, 71–84.
4. Lancaster W, Fidelity L, People, land and water in the Arab Middle East. Taylor & Francis. ISBN 978-90-5702-322-4, 1999.
5. Williams JL, Genetic control of Meat Quality Traits. F. Toldr´ a (ed.), Meat Biotechnology ©Springer Science Business Media, LLC. 2008.
6. Nowak M, Rolakalpain w procesiekruszeniemięsa. Żywność. Nauka. Technologia. Jakość 1, 2005, 5–17.
7. Byun SO, Zhou H, Forrest RH, Frampton CM, Hickford JG, Association of the ovine calpastatin gene with birth weight and growth rate to weaning, Anim. Genet, 39, 2008, 572-573.
8. Palmer BR, Su HY, Roberts N, Hickford JG, Bickerstaffe R, Single nucleotide polymorphisms in an intron of the ovine calpastatin gene, *Animal Biotechnology*, 11, 2000, 63–67.
9. Zhou H, Hickford JG, Gong H, Polymorphism of the ovine calpastatin gene. Molecular and Cellular Probes, 21, 2007, 242–244. http://www.labimaging.com/servlet/engine/home/start.html.
10. Sumantri C, Diyono R, Farajallah A, Inounu I, Polymorphism of calpastatingene and its effect on body weight of local sheep’s. JITV, 13, 2008, 117-126.
11. Gábor M, Trakovická A, Miluchová M, Analysis of polymorphism of CAST gene and Clpg gene in sheep by PCR-RFLP method. J. Zoot şiBiotehnol, 42, 2009, 470-476.
12. Cockett NE, Smit MA, Bidwell CA, Segers K, Hadfield TL, Snowder GD, Georges M, Charlier C, The callipyge mutation and other genes that affect muscle hypertrophy in sheep. Genet. Sel. Evol, 37(1), 2005, S65–S81.
13. Cockett NE, Jackson SP, Shay TL, Nielsen DM, Moore SS, Steele MR, Barendse W, Green RD, Georges, M, Chromosomal localization of the *callipyge*gene in sheep (*Ovisaries*) using bovine DNA markers, *Proc. Natl. Acad. Sci.,* 91, 1994, 3019–3023.
14. Freking BA, Keele JW, Beattie CW, Kappes SM, Smith TPL, Sonstegard TS, Nielsen MK, Leymaster KA, Evaluation of the ovine callipyge locus: I. Relative chromosomal position and gene action, *J. Anim. Sci.,* 76, 1998, 2062–2071.
15. Cockett NE, Jackson SP, Shay TL, Farnir F, Berghmans S, Snowder GD, Nielsen DM, Georges M, Polar overdominance at the ovine callipyge locus, Science, 273, 1996, 236.
16. Miller SA, Dykes DD, Polesky HF, A simple salting out procedure for extracting DNA from human nucleated cells. Nucl. Acids Res., 16, 1988, 12-15.
17. Suleman M, Saeed U, Khan M, Naeem R, Muhammad Y, Abdullah S, RafaqatIshaq A, Calpastatin (*CAST*) gene polymorphism in Kajli, Lohi and Thalli sheep breeds. African Journal of Biotechnology, 11(47), 2012, 10655-10660.
18. Yeh FC, Boyle T, Rongcai Y, Ye Z, Xian JM, POPGENE, Version 1.31. A Microsoft Window Based Free Ware for Population Genetic Analysis. University of Alberta, Edmonton 1999.
19. Palmer BR, Robert N, Hickford JGH, Bickerstaffe G, Rapid Communication: PCR-RFLP for *Msp*I and *Nco*I in the ovine calpastatin gene, *J Anim Sci*, 76, 1998, 1499-1500.
20. Chung HY, Davis MS, Hines HC, Effect of calpain and calpastatin genotypes on growth of Angus Bulls. Ohio State University Extension Research Bulletin: Special Circular. 2001, 181-201.
21. Nassiry MR, Mojtaba T, Ali J, Mahdi S, Saheb FF, Calpastatin polymorphism and its association with daily gain in Kurdi sheep. J. Iran. Biotechnol. 4, 2006, 188-192.

22. Shahroudi FE, Nassiry MR, Valizadh R, Moussavi AH, Pour MT, Ghiasi H, Genetic polymorphism at MTNR1A, CAST and CAPN loci in Iranian Karakul sheep. J. Iran. Biotechnol. 4, 2006, 117-122.

23. Qanbari S R, Osfoori M P, Eskandari N, Introgression of major genes into elite breeding flock of afshari sheep: a preliminary evaluation of polymorphism content and applicability of marker data. Biotechnology. 6, 2007, 513-519.

24. Jackson S P, Miller M F, Green R D, Phenotypic characterization of Rambouillet sheep expressing the callipyge gene: I. Inheritance of the condition and production characteristics, *J. Anim. Sci.*, 75, 1997, 14.

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