IJAS Iranian Journal of Applied Animal Science

The Calpastatin Gene Polymorphism Study and Its Effect on Early Body Weight Traits in Zandi Sheep

Research Article

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Received on: 4 Jan 2019
Revised on: 29 Jul 2019
Accepted on: 15 Aug 2019
Online Published on: Jun 2020

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Online version is available on: www.ijas.ir

ABSTRACT

The goal of this study was investigation of the calpastatin gene polymorphism and its effect on early body weight traits in Zandi sheep by polymerase chain reaction restriction fragment length polymorphism (PCR-RFLP) method. Calpastatin is the main inhibitors of calpain-calpastatin enzyme system that play an important role in regulating the protein regeneration process. Genomic DNA was extracted by whole blood samples from 124 randomly selected Zandi breed sheep using salting out method. Birth weight, weight on 150 days old and daily weight gain from birth to 150 days old were recorded. The exon and intron 1 of ovine calpastatin gene were amplified to produce a 622 bp fragment. The PCR products were digested with MspI restriction enzyme and electrophoresised on agarose gel. Results showed 80.6% and 19.4% allele frequencies for A and B alleles and 65.32%, 4.04% and 30.64% genotype frequencies for AA, BB and AB genotypes, respectively. X^2 and G^2 tests showed the Hardy-Weinberg equilibrium for studied locus. The effect of calpastatin genotype on growth traits was insignificant, but genotype BB had the best performance among all the three traits, which indicates the better performance of normal allele (B) than mutant allele (A) for these traits.

KEY WORDS body weight traits, MspI, PCR-RFLP method, polymorphism of calpastatin gene, Zandi sheep.

INTRODUCTION

The advancement of biotechnology in recent years has greatly contributed to livestock breeding. This advancement has led to the identification of many controlling genes, such as those controlling meat production and quality, through combining quantitative and molecular genetics. Calpains are calcium-dependent proteases which play an important role in regulating the protein regeneration process that occurs every day in the body through cleaving the skeletal muscle myofibrils (Li *et al*. 2009). The calpain-calpastatin system is important in the growth rate of skeletal muscle and generally in the growth rate of body. In fact, increased activity of calpastatin leads to the inhibition of calpains and decreased decomposition of meat proteins and hence increased rate of the body growth (Kania, 2012). The calpastatin gene is located on the sheep's chromosome 5 and consists of 36 exons and 35 introns. Polymorphism of the calpastatin gene was first identified by Palmer *et al*. (1998) through PCR-RFLP and was introduced as an important tool for selecting sheep for improving meat quality. Over the next few years, different researchers also confirmed the polymorphism of this gene. Mohamadi *et al*. (2008) examined the polymorphism of the calpastatin gene in Arabic sheep in the south of Iran through PCR-RFLP and showed the calpastatin gene polymorphism. Bahrampoor (2008) examined the polymorphism of the calpastatin gene and determined the gene and genotype frequency in four herds

of Kermani sheep, and identified three genotypes of NN, MN, and MM through RFLP, and showed that this site is highly polymorphic.

The calpain-calpastatin system is a proteolytic protein complex that plays a major role in muscle development and meat tenderness, and its existence has been proven in all muscle cells (Edyta *et al*. 2002).

Several studies have reported that calpastatin activity is highly heritable, and selection based on calpastatin activity can improve muscle growth and meat tenderness (Palmer *et al*. 1998).

Fakhr Kazemi and Nasiry (2007) investigated the polymorphism of this gene in Sistani cattle through RFLP and observed that MM genotype results in a better weight gain at age 0-3 months and 9-12 months.

Investigating the relationship between calpastatin gene polymorphism at two sites of exon 6 and exon and intron 1 both through single-strand conformation polymorphism (SSCP) with meat tenderness, carcass weight, and carcass components weight in 150 sheep of 10-12 months from 70 different New Zealand herds led to the observation of alleles a, b, c, and d in exon 6 and alleles A, B, C, and D in exon and intron 1. Finally, a significant association was found between the three alleles A, B, and a with the sirloin weight, so that one of these alleles resulted in an increase of 15% in the sirloin weight.

Nassiry *et al*. (2006) studied the relationship of calpastatin gene polymorphism with early growth traits such as daily weight gain and weaning weight in Kurd sheep through PCR-SSCP and reported a significant effect of the genotype on these traits.

Hassankhan *et al*. (2012) investigated the polymorphism of Calpastatin with the daily weight gain in Balkhi, Cajali sheep and Beatle goat through PCR-RFLP and reported more daily weight gain for those with heterozygous genotypes.

In contrast, in the study of Sutikno *et al*. (2011) on native sheep of Indonesia through PCR-RFLP using restricting enzymes MspI and NcoI, none of the genotypes had significant effect on body weight gain. Therefore, according the importance of Zandi breed sheep in Iran, the present experiment was designed to determine the existence and frequency of this gene and the relation between its polymorphism and early growth traits in Zandi sheep.

MATERIALS AND METHODS

Body weight measurements

Birth weight, weight on day 150 of age and daily weight gain of male and female Zandi sheep during days 1-150 were recorded.

Sample collection and analyses

Random blood samples were selected from 124 male and female Zandi sheep (150±5 days-old) from jugular vein in the 5 mL vacuum tubes containing ethylene diaminete traacetic acid (EDTA) gel. Zandi breed sheep belonged to a semi-industrial husbandry in Qom province, iran. They have the same breeding system and nutrition condition. Then samples immediately frozen in liquid nitrogen and stored at −70 ˚C until further analysis for DNA extraction. DNA extraction done by salting out method. Electrophoresis on 0.8% agarose gel and spectrophotometer was used for investigating quality and quantity of DNA extracted. The exon and intron 1 of ovin calpastatin gene were amplified to produce a 622 bp fragment. PCR amplification was performed using specific primer pairs (CastF:5'-TGGGGCCCAATGACGCCATCGATG-3'; CastR:5'-GGTGGAGCAGCACTTCTGATCACC-3') according to Palmer *et al*. (1998) method. PCR was performed using a buffer PCR 1X, 2.5 µL/$MgCL_2$, 1.5 mM/each primer, 0.25 µM/dNTPs, 200 µM/taq DNA polymerase, 1 u/ovine gnomic DNA 50-100 ng and ddH_2O up to a total volume of 25 µL. Amplification of calpastatin gene was performed for 35 cycles, which consisted of an initial denaturation step (94 ˚C, 2 min), denaturation cycle (95 ˚C, 1 min), annealing (62 ˚C, 1 min), extension (72 ˚C, 2 min) and final extension (72 ˚C, 10 min). The PCR products were separated by 1.5% (w/v) agaros gel electrophoresis. The amplified fragment of calpastatin was digested with MspI. Enzyme buffer 1X, 1 µL; MspI, 5 unit/µL; PCR production 5 µl and ddH_2O 3 µL according to the manufacturers instruction for 12-14 h at 37 ˚C. At the end, the digestion products were electrophoresed on 2% agarose gel.

Statistical analysis

Statistical analyses of genotype, alleles frequencies and the Hardy-Weinberg equilibrium for population was carried out by POPGENE 3.2 and SAS for Windows version 9.2 (SAS, 2004) with the following statistical models:

Statistical model for birth weight trait:

$$y_{ijkl} = \mu + sex_i + year_j + G_k + e_{ijkl}$$

Where:
y_{ijk}: mean value of the trait.
μ: effect of mean.
sex_i: effect of sex (i=1 and 2).
$year_j$: effect of year (i=1, 2, 3, 4, 5 and 6).
G_k: effect of genotype (k=1, 2 and 3).
e_{ijkl}: random error.

Statistical model for 5 months old's weight and daily weight gain traits (since birth to 5 months old):

$$y_{ijklmn} = \mu + sex_i + b_1 bw_j + b_2 Dim_k + year_l + G_m + e_{ijklmn}$$

Where:
y_{ijklm}: mean value of the trait.
μ: effect of mean.
sex_i: effect of sex (i=1 and 2).
b_1: regression coefficient of bw_j.
bw_j: birth weight auxiliary variable.
b_2: regression coefficient of Dim_k.
Dim_k: auxiliary variable of days number to the second weighing.
$Year_l$: effect of year (L=1, 2, 3, 4, 5 and 6).
G_m: effect of genotype (m=1, 2 and 3).
e_{ijklmn}: random error.

RESULTS AND DISCUSSION

Calpastatin gene polymorphism study

The PCR product on a 1.5% agarose gel is presented in Figure 1.

The results show the completely sharp bands of the calpastatin gene 622 bp fragment with no additional bands and contamination due to the specificity and proper annealing of the primers.

These results are consistent with the findings of Nanekarani *et al*. (2011) in Atabi sheep. In a study on Slovak native sheep, Szkudlarek-Kowalczk *et al*. (2011) and Gabor *et al*. (2009) replicated the 622 bp fragment using the same primers as this study; their results are consistent with the findings of the present research. Other regions of this gene have also been studied; for example, Kania (2012) replicated a 425 bp fragment of intron 12.

The results of PCR digestion by the MspI enzyme are shown in Figure 2.

The pattern of the bands on the gel can indicate the alleles A and B and the AA, BB, and AB genotypes; so that, if the 622 bp fragment is cut by MspI to two fragments of 336 bp and 286 bp, the allele is A and mutated; if it is not cut, the allele is B and not mutated; if only the 622 bp band is seen on the agarose gel, the genotype is BB; if the 336 bp and 286 bp bands are seen, the genotype is AA; and if all 3 bands are observed, the genotype is heterozygous (AB).

This result shows that the polymorphism were detected in CASTI segment as previously observed by Mohamadi *et al*. (2008), Szkudlarek-Kowalczk *et al*. (2011) and Suleman *et al*. (2012). Calpastatin gene and genotype frequency are shown in Figures 3 and 4 respectively. A and B allele frequencies were 80.6% and 19.4% respectively and the genotype frequencies were 65.32% For AA, 4.04% for BB and 30.64% for AB.

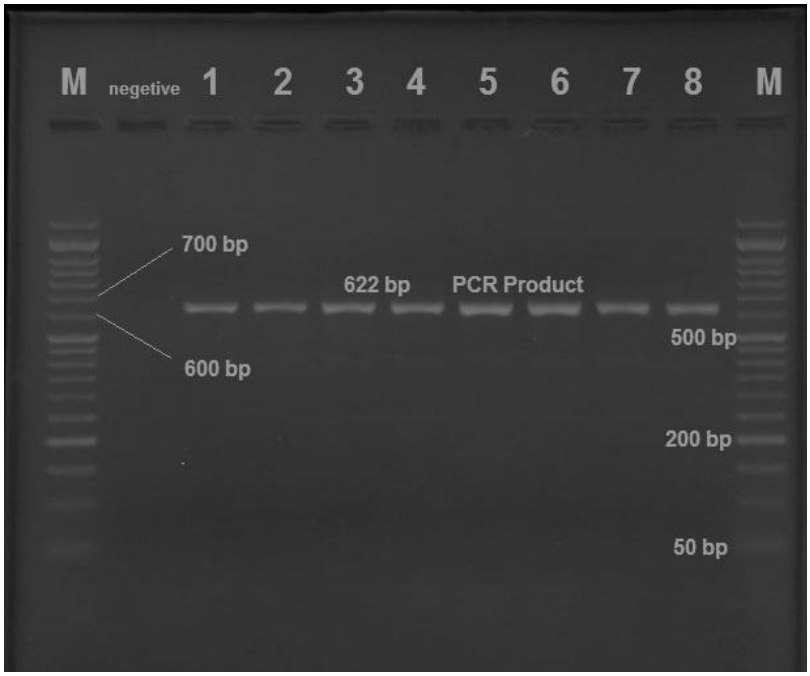

Figure 1 PCR products on 1.5% agarose gel (M=50 bp)

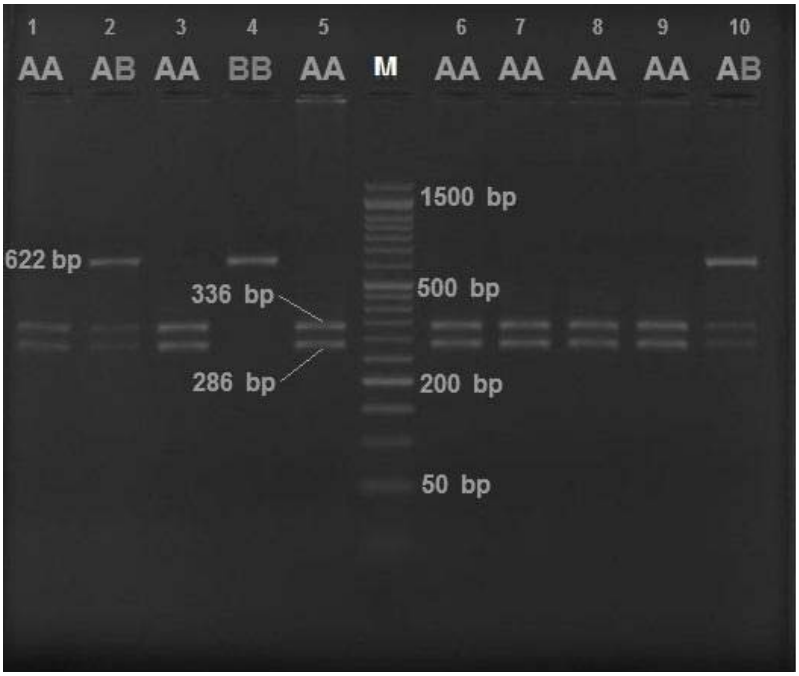

Figure 2 Digestion of PCR products by the MspI enzyme on 2% agarose gel (M=50 bp)

The results of this study for gene and genotype frequencies are very close to the findings of the previous studies on Iranian Ghara Gol sheep (Eftekhari Shahroudi *et al*. 2006), Kermani sheep (Bahrampoor, 2008), and Berrichon du Cher, IIe de France and Polish Merino sheep (Szkudlarek-Kowalczk *et al*. 2011).

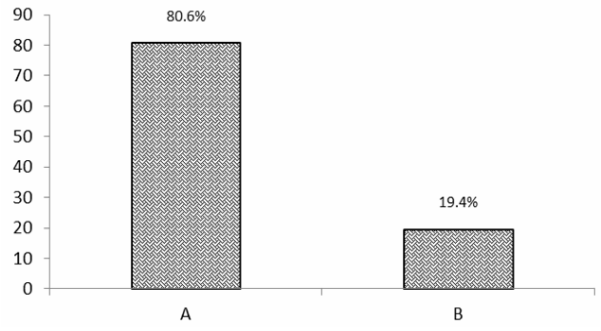

Figure 3 Allele frequency of calpastatin

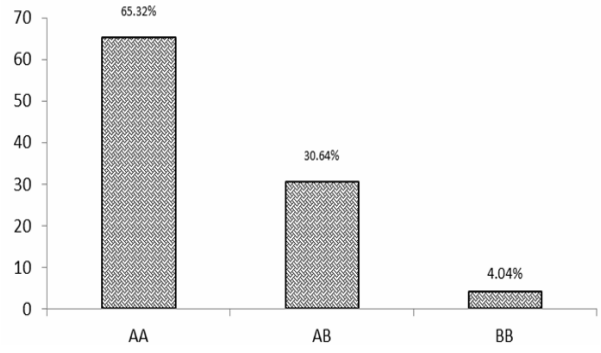

Figure 4 Genotype frequency of calpastatin

The Shannon Index and the heterozygosity value (Nei) in this study were 0.49 and 0.31, respectively, indicate a good variety of this breed in the population. The results of X^2 and G^2 tests for the Hardy-Weinberg equilibrium of studied locus as presented in Table 1. This indicates the lack of selection and breeding program in this herd. Given the point mutation at the site, it is in an equilibrium, therefore, mutation in this site is meaningless due to natural selection and absence of breeding programs, the same as the results of Hassankhan *et al*. (2012) and Ranjbari *et al*. (2012).

Effect of calpastatin genotype on growth traits

Despite a better performance of BB genotype for all the three growth traits, the effect of calpastatin genotype on the growth traits was insignificant (Table 2). Nikmard *et al*. (2012) did not find significant correlation between the calpastatin gene genotype and the growth and carcass traits in Afshari sheep using PCR-RFLP. Dehnavi *et al*. (2012) also reported insignificant effect of calpastatin gene on one-year old sheep weight by using both PCR-RFLP and PCR-SSCP techniques. In a study by Chung and David (2012) on Polypay, Targhee, and their crossbred sheep through PCR-RFLP, the effect of calpastatin genotype on weaning and post-weaning weights was also insignificant. However some study mentioned the significant effect of genotype in already.According to literature and the results of other researchers, it can be concluded that the effect of calpastatin genotype on growth traits decreases with the livestock aging, so that the effect of genotype was not important on the weights after 8-month old in none of the references.

Table 1 The Hardy-Weinberg equilibrium study by X^2 and G^2 tests

Genotype	O	E	$(O-E)^2/E$	Df	X^2	Probability (X^2)	G^2	Probability (G^2)
AA	81	80.56	0.0023					
AB	38	38.86	0.0193	1	0.0627	0.802224	0.0616	0.803930
BB	5	4.5	0.041					

Table 2 Effect of calpastatin genotype on growth traits (mean±standard error) (kg)

Calpastatin genotype	Birth weight	Weight on day 150	Daily weight gain (1-150)
AA	4.34±0.06	44.36±0.31	0.29±0.01
AB	4.33±0.07	44.87±0.40	0.29±0.01
BB	4.35±0.19	45.52±1.00	0.30±0.01
Pr > f	0.9850	0.2694	0.4228

In addition, the results of other methods showed that the effect of genotypes on traits was more significant by PCR-SSCP than PCR-RFLP, which may be due to more alleles and genotypes investigated and a better discovering of genome in this technique than PCR-RFLP. The body weight is a polygenic trait and it is difficult to relate the total variation of the trait with just single nucleotide polymorphism. Of course, the ability to control dystocia can be a positive aspect of insignificancy of the genotype effect on the birth weight for breeders.

CONCLUSION

The present study is about the effect of calpastatin gene pholymorphism on early growth trait in Zandi sheep as an important resource breed of sheep. According of our results for three recognized genotypes in exon and intron 1 in this population, there was acceptable polymorphism in the studied locus, but the Hardy-Weinberg equilibrium shows the absence of animal breeding programs for change the useful allele frequency in the studied samples and it could be confirm by the conventional breeding system for this sheep. We found insignificant effect on studied growth traits but the more study on a bigger population size and for other economic traits can be purposed.

ACKNOWLEDGEMENT

The authors thank the Islamic Azad University, Karaj Branch for Research Laboratory Agriculture Faculty teamwork.

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