Calpastatin Polymorphism in Barki Lambs and their Effects on Growth and Carcass Traits

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Abstract: The objectives of this study were to identify the allelic and genotypic polymorphisms of the calpastatin gene in Barki sheep and to assess the effect of these polymorphisms on growth and carcass traits of Barki sheep. Forty two males of Barki lambs were phenotyped for growth (Birth weight, pre-weaning daily gain, weaning weight, post-weaning daily gain and marketing weight) and carcass (hot carcass weight, dressing%, neck%, shoulder%, racks% , loin%, flanks%, legs%, tail%, 9-10-11 rib cut weight, lean meat%, fat% and bone%) traits. The polymerase chain reaction- restricted fragment length polymorphism (PCR-RFLP) tool was used to identify the allelic and genotypic polymorphism in the calpastatin gene. The associations between the variation in calpastatin gene (calpastatin genotype and the absence/presence of each calpastatin allele in animal genotype) and the studied traits were tested using the general linear model for the version 19 of SSCP software. Four alleles (M, N, O and P with frequencies of 0.62, 0.13, 0.10 and 0.15, respectively), and six genotypes (MM, NN, OO, MP, NO and OP with frequencies of 0.55, 0.05, 0.05, 0.14, 0.16 and 0.05, respectively) were detected. The association of calpastatin genotype was significant with lean meat % ($P < 0.01$) and fat % ($P < 0.05$). The presence of allele O was significantly associated with the higher ($P < 0.001$) lean meat % and the lower ($P < 0.01$) fat%, however the presence of allele M was significantly associated with the lower ($P < 0.01$) lean meat% and higher fat% ($P < 0.05$). Finding the association of variation in calpastatin gene with growth and carcass traits in Barki sheep may be useful to get less carcass fat and great carcass lean meat.

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1. Introduction

Growth and carcass traits are economically important and selection-responsive traits in sheep. Traditional selection for these traits has been based on estimating breeding value and remains difficult and expensive. Current technologies enable scientists to improve the accuracy and efficiency of traditional selection by applying molecular markers. This can be done through marker assisted selection which combines information on genetic polymorphisms with data on phenotypic variation among individuals (**Hines et al., 1998**).

One of the ways of identifying molecular marker that determine a given trait group is to evaluate DNA polymorphism in a candidate gene underlying the trait and contributing to a phenotype. These gene products participate in the physiological processes leading to trait expression, while polymorphic forms of the gene are associated with trait variation **(Gregula-Kania, 2012)**. This approach is a powerful method for finding the molecular marker loci responsible for genetic variation in the traits of interest (**Rothschiled and Soller, 1997**)

In sheep, skeletal muscles constitute about 25 % of body weight (**Lee et al., 2001**). The rate and extent of skeletal muscle growth depend mainly on three factors: rate of muscle protein formation, rate of muscle protein degradation and the number and size of skeletal muscle cells (**Khederzadeh, 2011).**

In mammals, calpain-calpastatin system plays a crucial role in the growth and development of skeletal muscles **(Goll et al., 2003**). The activity of calpastatin is highly correlated with muscle growth rate through its role to inhibit the activity of calpains that result in decreasing the rate of protein degradation **(Gregula-Kania, 2012)**. The balance between the rate of protein formation and the rate of protein degradation determine the growth rate of muscles in live animals.

In growing animals, synthesis of muscle tissues is faster than breakdown resulting in increasing muscle mass. This is associated with the increase in activity of the calpastatin and the decrease in calpain activity (**Goll et al., 1998**). Conversely, in slaughtered animals, the activity of calpains highly exceeds the activity of calpastatin resulting in high degradation rate of muscles which cause tenderizing of meat.

Many studies had focused on the genetic variation which occurred at the calpastatin gene in sheep (**Tahmoorespour, 2005; Zhou et al., 2007; Nanekarani et al. 2011a&b; Dehnavi et al., 2012;**

Nikmard et al., 2012; Yilmaz et al., 2014a) and cattle (**Chung et al., 2001; Nassiry et al., 2006; Edyta et al., 2002; Byun et al., 2009; Sutikno et al., 2011; Yousefi and Azari, 2012; Yang, 2013**) using polymerase chain reaction-restricted fragment length polymorphism (PCR-RFLP), polymerase chain reaction-single strand conformational polymorphism (PCR-SSCP) or single nucleotide polymorphism (SNP) techniques. All studies used these techniques approved a high degree of polymorphisms at this gene.

Associations between the calpastatin genotype and growth and carcass traits of meat animals had been reported. In sheep, significant differences among calpastatin genotypes were found for pre-weaning daily gain in Kurdi lambs **(Nassiry et al., 2006),** post-weaning average daily gain, thickness and skin+backfat thickness values of loin eye muscle in Kivircik Lambs **(Yilmaz et al., 2014b)**. However in cattle, calpastatin genotypes were found significantly associated with skeletal muscle mass **(Edyta et al., 2002)**; percentage of kidney, pelvic and heart fat **(Chung et al., 2011)**; carcass fat yield **(Schenkel et al, 2006)**, tenderness traits **(Casas et al., 2006)** and reproductive traits and length of productive life **(Morris et al., 2006)**.

From the previous, the calpastatin gene, which located on the ovine chromosome 5, is considered as an important candidate gene for growth and carcass traits in Barki sheep. The objective of this study was to determine the allelic and genotypic polymorphism of ovine calpastatin gene and to test their association with growth and carcass traits in Barki sheep.

2. Materials and Methods

Data collection

This study was carried out at Maryout Research Station, which is one of the experimental stations of Desert Research Center. Forty two males of Barki lambs were phenotyped for growth traits (birth weight, pre-weaning daily gain, weaning weight, post-weaning daily gain and marketing weight).

Blood samples were drawn from the jugular vein into 5 ml heparinized tubes. These samples stored at – 80 ^{0}c for several months, whereupon genomic DNA external with the use of phenol/chloroform **(Sambrook et al., 1989)**.

At the age of 9 months, slaughtering was carried out by serving the carotid artery and jugular vein. After slaughtering and skinning, all abdominal and thoracic offal's were removed to obtain the hot carcass weight. All carcasses were chilled at 4 ºC for 24 h to evaluate the chilled carcass weight. Each chilled carcass was dissected into seven parts (neck, shoulder, racks, loin, flanks, legs and tail) according to the norms of the Egyptian wholesale mutton cuts as described by **Hamada (1976)**. The seven cuts were weighed to calculate the percentages of the chilled carcass cuts. Also, the 9-10-11 rib cut of each animal was separated into its physical components (lean, fat and bone) and weighed to be expressed as percentages of the weight of the whole rib cut.

Genotyping determination

Two specific primers were used to amplify 565 pb from exon 1 of the ovine calpastatin gene. The sequences of these two primers were based on **Khederzadeh (2011**), and were as follow: Forward: 5`- CCTTGTCATCAGACTTCACC - 3` and Reverse: 5`- ACTGAGCTTTTAAA-GCCTCT – 3`. PCR carried out in a total reaction volume of 25 ul, containing 2.5 ul of 10x PCR buffer. 1.5 mM of MgCl2, 200 uM of dNTP, 2 uM of each primer, 50 ng of genomic DNA and 1 U of Taq DNA polymerase. The amplification conditions were as follows: initial duration at 95 ^{0}c for 5min, followed by 35 cycles of denaturation at 95 ^{0}c for 40 sec, annealing at 65 ^{0}c for 1 min and extension at 72 ^{0}c for 2 min. A final 10 min extension step was completed at 72^{0}c. PCR products of 5 ul were digested with 10 units of MSpI restriction enzyme (Ferments) at 37 ^{0}c for 5 hours.

Capillary electrophoresis

High-resolution capillary electrophoresis was performed using a QIAxcel® DNA high resolution gel cartridge (Qiagen). A QX DNA Size Marker (Qiagen) with 12 fragment sizes ranging in size from 15 to 600 bp was used to size PCR products. 0.1 µl of each digested PCR product was injected onto a cartridge for analysis. A QX Alignment Marker (Qiagen) was injected onto the cartridge with each PCR product. The retention time of the PCR fragments relative to the 15-bp and 600-bp. QX Alignment Marker fragments was calculated using the BioCalculator software (Qiagen). The PCR product sizes were then determined by comparing the retention time with the QX DNA Size Marker. The BioCalculator software produces digital gel images for fragment analysis.

Statistical analysis

Allelic and genotypic frequencies of calpastatin gene were calculated using simple gene counting method **(Falconer and Mackey, 1996)**. Hardey-Weinberg equilibrium was tested by comparing expected and observed genotypic frequencies using χ^2 test. The population would be considered to be in Hardy-Weinberg equilibrium if it failed the χ^2 test at the level of 0.05.

Associations of variation at calpastatin gene with growth and carcass traits were determined by analysis of variance of quantitative traits. General Linear Model (GLM) procedure in SAS (1990) was used to perform the analysis.

Fixed effect of variation at calpastatin gene (calpastatin genotype or the absence/presence of each allele in animal genotype), and random effect of sire were included as independent variables in the linear model. In the models assessing the effect of variation at calpastatin gene on weaning weight and pre-weaning daily gain, weaning age was included as a covariate. Where significant, these were further explored using pairwise comparison (Duncan test; P≤ 0.05).

The general linear model was:

$$Y_{ijk} = \mu + G_i + S_j + e_{ijk}$$

Where Y_{ijk} = observed value; μ = overall mean for each trait; G_i = fixed effect of the variation at calpastatin gene; S_i = random effect of sire and e_{ijk} = random error.

3. Results and Discussion

Allelic and genotypic frequencies

The result of RFLP analysis using the capillary electrophoresis (Fig. 1), in the examined lambs, showed a total of six RFLP band patterns (MM, NN, OO, MP, NO and OP with frequencies of 0.55, 0.05, 0.05, 0.14, 0.16 and 0.05, respectively), representing four alleles (M, N, O and P with frequencies of 0.62, 0.13, 0.10 and 0.15, respectively). The previous results of **Khederzadeh (2011)**, **Yilmaz et al. (2014b), Ata and Cemal (2013),** that used the agarose gels, detected only two alleles (M & N), however using the capillary electrophoresis in this study detected another two alleles (O&P). This result might due to a genetic difference between Barki sheep and other breeds at this locus, or to the high sensitivity of the capillary electrophoresis tool in identifying the variation in genome sequence.

Effect of sire on the studied traits

Sire had significant effect (P < 0.05) on the post-weaning daily gain.

Effect of the calpastatin genotype on the studied traits

The presented results in Table (1) showed that the calpastatin genotype had significant effect (P < 0.05) on fat percentage and high significant effect (P < 0.01) on lean meat percentage. There were no association between the calpastatin genotype and the other studied traits. Also, the least square means (LSM) showed that, lambs with the genotypes OO and OP had higher lean meat percentage and lower fat percentage; however lambs with the genotype MP had lower lean meat percentage and higher fat percentage. Like our results, the calpastatin genotypes were found to affect the carcass fat yield in beef cattle **(Schenkel et al, 2006)** and fat thickness in pigs (Kurył et al., 2003).

Effect of the absence / presence of alleles in the calpastatin genotype on the studied traits

The results of the second set of analysis concerned the effect of the absence / presence of alleles in the calpastatin genotype on growth and carcass traits are presented in Tables (2&3). The presence of allele M was associated with lower lean meat percentage (P < 0.01) and higher fat percentage (P < 0.05), however the presence of allele O was associated with higher lean meat percentage (P < 0.001) and lower fat percentage ((P < 0.01).

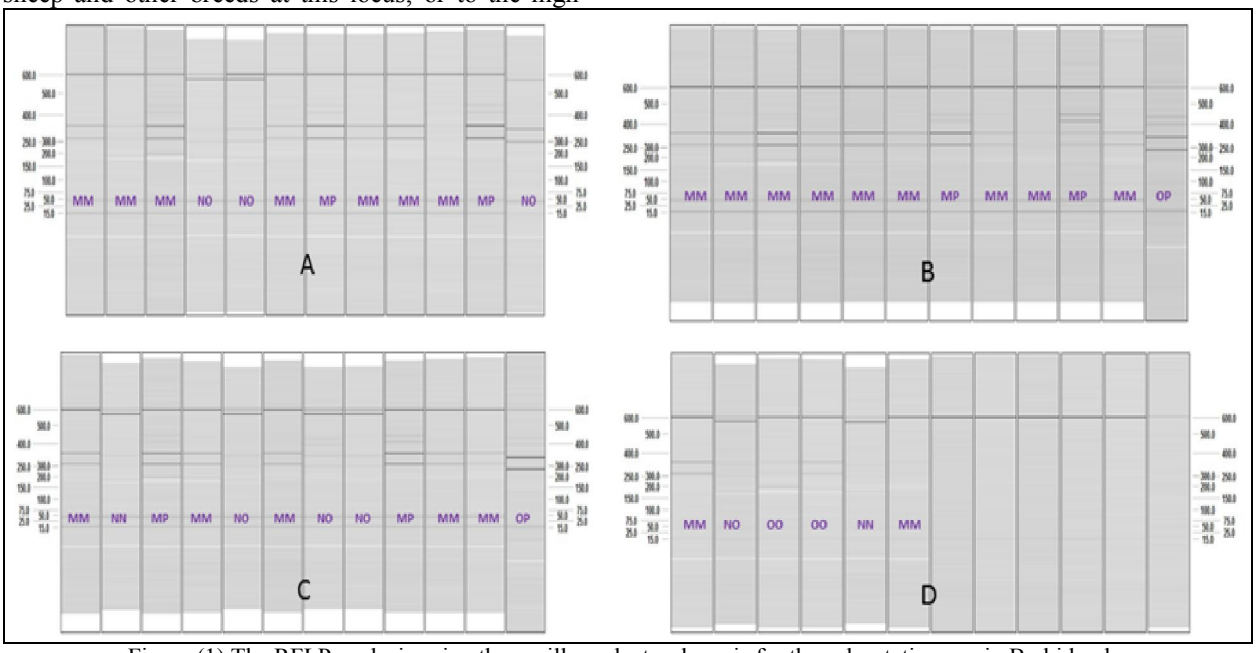

Figure (1) The RFLP analysis using the capillary electrophoresis for the calpastatin gene in Barki lambs

Table (1) Least square means and standard errors for growth and carcass traits according to the calpastatin genotypes in Barki lambs

Traits	LSM ± SE						P-values
	MM (23)	NN (2)	OO (2)	MP (6)	NO (7)	OP (2)	
Growth traits							
Birth weight (Kg)	3.48 ± 0.11	3.50 ± 0.50	3.25 ± 0.25	3.67 ± 0.21	3.42 ± 0.15	3.75 ± 0.75	NS
Weaning weight (Kg)	20.32 ± 0.67	18.75 ± 3.75	24.75 ± 1.25	21.83 ± 1.41	20.41 ± 1.46	15.75 ± 5.75	NS
ADG1	187.48 ± 8.3	171.34 ± 48.8	250.91 ± 26.1	197.79 ± 17.5	184.79 ± 16.9	133.41 ± 61.9	NS
ADG2	79.67 ± 6.05	82.04 ± 33.48	91.33 ± 15.12	64.15 ± 11.47	70.65 ± 9.23	84.77 ± 18.22	NS
Marketing weight (Kg)	41.76 ± 1.77	40.75 ± 12.25	49.00 ± 5.00	39.20 ± 3.78	39.60 ± 2.02	38.75 ± 10.25	NS
Carcass traits							
Hot carcass weight (Kg)	19.01 ± 1.00	18.16 ± 5.81	22.26 ± 2.71	18.00 ± 2.32	17.80 ± 1.05	17.63 ± 5.35	NS
Dressing %	45.18 ± 0.49	44.28 ± 0.96	45.34 ± 0.90	45.24 ± 2.48	44.86 ± 0.38	45.00 ± 1.92	NS
Neck %	7.69 ± 0.57	6.33 ± 0.62	6.95 ± 0.07	9.15 ± 2.06	7.43 ± 0.34	7.43 ± 1.26	NS
Shoulder %	19.94 ± 0.28	21.38 ± 0.90	18.10 ± 0.37	20.74 ± 1.05	19.81 ± 0.61	20.23 ± 0.60	NS
Rack %	23.83 ± 0.80	24.25 ± 0.25	24.98 ± 0.70	20.87 ± 2.76	24.36 ± 0.25	24.88 ± 0.11	NS
Loin %	6.36 ± 0.21	6.83 ± 1.06	7.69 ± 0.38	6.75 ± 0.90	6.47 ± 0.39	6.56 ± 0.77	NS
Flank %	5.32 ± 1.36	4.10 ± 0.27	4.10 ± 0.52	9.08 ± 4.87	4.37 ± 0.31	3.78 ± 0.61	NS
Leg %	33.72 ± 1.34	34.91 ± 0.62	33.77 ± 0.32	27.93 ± 5.09	34.22 ± 0.75	34.47 ± 0.30	NS
Tail %	2.90 ± 1.99	2.17 ± 0.17	4.40 ± 0.42	7.22 ± 6.93	3.31 ± 0.44	2.64 ± 0.68	NS
9-10-11 rib wt	587.0 ± 37.7	596.8 ± 166.3	755.5 ± 156.2	450.5 ± 85.1	531.9 ± 22.7	488.1 ± 17.0	NS
Lean-meat %	**44.27 ± 1.44^{b}**	**42.15 ± 2.14^{b}**	**58.99 ± 1.71^{a}**	**39.72 ± 4.79^{b}**	**48.87 ± 2.04^{ab}**	**59.82 ± 1.40^{a}**	**
Fat %	**21.46 ± 1.12^{ab}**	**25.17 ± 3.96^{a}**	**12.44 ± 0.41^{bc}**	**23.10 ± 2.57^{a}**	**18.81 ± 2.17^{abc}**	**9.53 ± 2.78^{c}**	*
Bone %	29.58 ± 1.39	30.60 ± 2.79	26.75 ± 0.98	25.34 ± 4.91	29.37 ± 0.39	28.47 ± 0.26	NS

NS refers to non-significant; * refers to significance at (P < 0.05); ** refers to significance at (P < 0.01).

Table (2) Least square means and standard errors for growth traits according to the absence/ presence of calpastatin alleles in Barki lambs

Trait	Allele being assessed	LSM ± SE				P-value
		Allele absent	n	Allele present	n	
Birth weight	M	3.42 ± 0.13	13	3.52 ± 0.10	29	NS
	N	3.52 ± 0.09	33	3.39 ± 0.13	9	NS
	O	3.52 ± 0.09	31	3.41 ± 0.14	11	NS
	P	3.44 ± 0.08	34	3.69 ± 0.21	8	NS
Pre-weaning daily gain	M	179.15 ± 16.45	13	189.61 ± 7.46	29	NS
	N	189.92 ± 8.03	33	173.37 ± 15.98	9	NS
	O	188.43 ± 7.38	31	180.57 ± 18.41	11	NS
	P	187.48 ± 7.61	34	181.70 ± 20.33	8	NS
Weaning weight	M	19.61 ± 1.34	13	20.63 ± 0.60	29	NS
	N	20.59 ± 0.65	33	19.33 ± 1.33	9	NS
	O	20.51 ± 0.60	31	19.77 ± 1.51	11	NS
	P	20.32 ± 0.60	34	20.31 ± 1.80	8	NS
Post-weaning daily gain	M	76.07 ± 6.95	13	76.46 ± 5.39	29	NS
	N	77.86 ± 4.89	33	70.74 ± 8.77	9	NS
	O	76.82 ± 5.28	31	74.98 ± 6.88	11	NS
	P	77.99 ± 4.76	34	69.30 ± 9.68	8	NS
Marketing weight	M	40.18 ± 2.53	13	41.23 ± 1.59	29	NS
	N	41.55 ± 1.52	33	38.53 ± 2.78	9	NS
	O	41.20 ± 1.59	31	40.07 ± 2.52	11	NS
	P	41.33 ± 1.46	34	39.09 ± 3.38	8	NS

NS refers to non-significant; * refers to significance at (P < 0.05); ** refers to significance at (P < 0.01); *** refers to significance at (P < 0.001)

Table (3) Least square means and standard errors for carcass traits according to the absence/presence of calpastatin alleles in Barki lambs

Trait	Allele being assessed	Mean ± SE				P-value
		Allele absent	n	Allele present	n	
Hot carcass weight	M	18.06 ± 1.25	13	18.80 ± 0.91	29	NS
	N	18.94 ± 0.85	33	17.23 ± 1.35	9	NS
	O	18.76 ± 0.89	31	18.05 ± 1.27	11	NS
	P	18.73 ± 0.79	34	17.91 ± 1.98	8	NS
Dressing %	M	44.73 ± 0.35	13	45.19 ± 0.61	29	NS
	N	45.19 ± 0.54	33	44.53 ± 0.35	9	NS
	O	45.13 ± 0.57	31	44.81 ± 0.39	11	NS
	P	45.02 ± 0.35	34	45.18 ± 1.85	8	NS
Neck%	M	7.28 ± 0.27	13	7.99 ± 0.61	29	NS
	N	7.89 ± 0.54	33	7.32 ± 0.33	9	NS
	O	7.88 ± 0.57	31	7.46 ± 0.28	11	NS
	P	7.55 ± 0.39	34	8.72 ± 1.55	8	NS
Shoulder%	M	19.84 ± 0.40	13	20.10 ± 0.31	29	NS
	N	19.99 ± 0.28	33	20.14 ± 0.48	9	NS
	O	20.19 ± 0.29	31	19.56 ± 0.40	11	NS
	P	19.88 ± 0.24	34	20.61 ± 0.78	8	NS
Rack%	M	24.55 ± 0.16	13	23.22 ± 0.85	29	NS
	N	23.43 ± 0.75	33	24.38 ± 0.17	9	NS
	O	23.29 ± 0.80	31	24.60 ± 0.18	11	NS
	P	24.04 ± 0.54	34	21.88 ± 2.13	8	NS
Loin%	M	6.67 ± 0.27	13	6.44 ± 0.24	29	NS
	N	6.52 ± 0.22	33	6.47 ± 0.32	9	NS
	O	6.46 ± 0.23	31	6.64 ± 0.28	11	NS
	P	6.47 ± 0.17	34	6.70 ± 0.67	8	NS
Flank%	M	4.20 ± 0.17	13	6.10 ± 1.45	29	NS
	N	5.84 ± 1.28	33	4.31 ± 0.21	9	NS
	O	5.97 ± 1.36	31	4.21 ± 0.20	11	NS
	P	4.98 ± 0.91	34	7.75 ± 3.67	8	NS
Leg%	M	34.33 ± 0.35	13	32.52 ± 1.51	29	NS
	N	32.72 ± 1.32	33	34.43 ± 0.51	9	NS
	O	32.68 ± 1.41	31	34.23 ± 0.41	11	NS
	P	33.91 ± 0.91	34	29.57 ± 3.87	8	NS
Tail%	M	3.10 ± 0.30	13	3.10 ± 0.33	29	NS
	N	3.00 ± 0.31	33	2.91 ± 0.35	9	NS
	O	3.11 ± 0.29	31	3.27 ± 0.33	11	NS
	P	3.12 ± 0.30	34	3.13 ± 0.30	8	NS
9-10-11 rib weight (gm)	M	564.90 ± 37.73	13	558.79 ± 35.54	29	NS
	N	566.43 ± 33.15	33	539.60 ± 33.84	9	NS
	O	561.24 ± 34.13	31	559.10 ± 38.66	11	NS
	P	584.38 ± 28.75	34	459.95 ± 62.72	8	NS
Lean meat %	**M**	**51.81 ± 2.05**	**13**	**43.33 ± 1.50**	**29**	**
	N	45.27 ± 1.61	33	48.44 ± 2.08	9	NS
	O	**43.25 ± 1.41**	**31**	**53.57 ± 1.97**	**11**	***
	P	46.24 ± 1.27	34	44.74 ± 4.81	8	NS
Fat%	**M**	**16.88 ± 1.82**	**13**	**21.80 ± 1.02**	**29**	*
	N	20.49 ± 1.10	33	19.50 ± 2.01	9	NS
	O	**22.01 ± 0.98**	**31**	**15.37 ± 1.71**	**11**	**
	P	20.41 ± 0.98	34	19.71 ± 2.95	8	NS
Bone%	M	28.93 ± 0.50	13	28.70 ± 1.48	29	NS
	N	28.57 ± 1.30	33	29.52 ± 0.58	9	NS
	O	28.82 ± 1.39	31	28.62 ± 0.39	11	NS
	P	29.39 ± 0.95	34	26.12 ± 3.63	8	NS

NS refers to non-significant; * refers to significance at ($P < 0.05$); ** refers to significance at ($P < 0.01$); *** refers to significance at ($P < 0.001$)

The two sets of analysis showed that the variation in calpastatin gene had no effect on all of the studied growth traits. Inconsistent with our findings, many studies revealed significant effect for the variation in calpastatin gene on birth weight in New Zealand Romney sheep **(Byun et al., 2008)**; pre-weaning daily gain in Kajil sheep **(Khan et al., 2012)** and Playpay, Targhee and crossbreed sheep **(Chung and Davis, 2012)** and daily gain from birth to marketing in Kurdi sheep **(Nassiry et al., 2006)** and Balkhi sheep **(Khan et al., 2012)**.

According to our results, the main association for the variation in the calpastatin gene was with lean meat and fat percentages of carcasses. Increasing the lean meat percentage of carcasses might due to the inhabitation effect of the calpastatin for the calpains **(Maki et al., 1988; Kawasaki et al., 1989),** which results in decreasing the rate of protein degradation and increasing the rate of protein synthesis in the skeletal muscles. The size of skeletal muscle mainly depends on the balance between the rate of degradation and the rate of synthesis for muscle protein.

Lean meat and fat percentages are the most important carcasses traits of lambs that provide optimal returns to the farmers as the higher lean meat percentage and the lower fat percentage is necessarily to produce high quality of meat that consumers prefer. These results could speculate that selection pressure for the correlated traits with this region may have reduced genotypic variation in this breed of sheep.

Conclusion

The obtained results suggested that the calpastatin genotype may be helpful for sheep breeders to reduce fat percentage and increase lean meat percentage of carcasses. These traits are critically related to the profitability of any production system of sheep. However, further studies concerning the polymorphisms and effects of calpastatin gene in Barki sheep and the other local breeds would be highly relevant to elucidate the influence of this genomic region in relation to lean meat and fat percentages and the other economic traits.

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