

*Original Research***Association of Calpastatin Polymorphism with Carcass and Meat Quality Traits of Bandur Sheep****S. Naveen Kumar^{1*}, M. R. Jayashankar², Wilfred Ruban³, R. M. Sreesujatha⁴ and Y. S. Roopa Devi⁵**¹Associate Professor, Department of Animal Genetics and Breeding, Veterinary College, KVAFSU, Hebbal, Bengaluru, Karnataka, INDIA²Retd. Professor, Department of Animal Genetics and Breeding, Veterinary College, KVAFSU, Hebbal, Bengaluru, Karnataka, INDIA³Associate Professor, Department of LPT, Veterinary College, KVAFSU, Hebbal, Bengaluru, INDIA⁴Research Associate, Livestock Research and Information Centre (Sheep), KVAFSU, K. R. Pete Road, Nagamangala, Karnataka, INDIA⁵Assistant Professor, Department of Veterinary Pathology, Veterinary College, Hassan, Karnataka, INDIA***Corresponding author:** navingen@gmail.com

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Abstract

The present study was aimed to determine the association of Calpastatin (CAST) gene polymorphism with carcass and meat quality traits of Bandur sheep. The genomic DNA extracted from 100 Bandur ram lambs was subjected to PCR using published primers to amplify ovine calpastatin gene (exon 1C/1D). The PCR-RFLP analysis of amplified CAST gene using restriction enzymes *MspI* and *NcoI* revealed two alleles viz., M and N with frequencies of 0.535 and 0.465, respectively. The genotypic frequencies were 0.24, 0.59 and 0.17 for MM, MN and NN, respectively. The carcass and meat quality traits were studied in 18 ram lambs belonging to the three CAST genotypes. No significant difference was observed between CAST genotypes and carcass traits. However, with respect to meat quality traits NN genotype had significantly lower Warner-Bratzler Shear Force value ($P < 0.001$) indicating higher tenderness and significantly higher total ash percentage ($P < 0.05$) than MM and MN genotypes. From the present study, it was concluded that ovine calpastatin gene may be considered as a possible candidate gene for meat tenderness in Bandur sheep.

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Introduction

Growth, carcass and meat quality traits are polygenic and considered as economically important traits in small ruminants meant for meat purpose. Selection of animals for enhanced growth and carcass conformation/ composition is of utmost significance for the breeders/ farmers and consumers. The recent gene based methods expedited the improvement of traits that are difficult to improve by traditional phenotype based methods. The gene based methods involves identifying the genes that have major effect on specific traits and determining the polymorphism in such genes *i.e.*, candidate genes which may be associated with the trait variation. The 'Calpain-Calpastatin System' (CCS) is constituted of three well characterized proteins, which include two ubiquitous Ca^{2+} dependent proteolytic enzymes (μ and m calpain), whereas, calpastatin is the third member capable of preventing more than one calpain molecule. The CCS plays a major role in skeletal muscle growth, developmental processes and meat production and quality (Goll *et al.*, 2003). The ovine calpastatin genes may be considered as potential candidate genes for growth, carcass and meat quality traits (Palmer *et al.*, 1999a).

Many research studies have confirmed the association of calpastatin polymorphism with growth and meat quality traits. Nassiry *et al.* (2006) in a study in Iranian Kurdi sheep found that AB genotype had higher daily weight gain, and Palmer *et al.* (1999a) reported that AC genotype was having increased live weight and carcass weight in the crossbred Dorset and Coopworth sheep. Bandur sheep, which is also known as Mandya sheep, is one of the best mutton breed of the State and the Country because of its conformation and unique organoleptic characteristics. But there is inadequate literature pertaining to carcass and meat quality traits and their possible association with calpastatin gene. In this regard, the present study intended to study the association of calpastatin polymorphism with carcass and meat quality traits in Bandur Sheep.

Materials and Methods

The research was conducted at Livestock Research and Information Centre (Sheep), KVAFSU, Nagamangala, Mandya district, Karnataka, India.

Experimental Animals

Genotyping study was undertaken on 100 Bandur ram lambs distributed over the villages of Malavalli taluk, Mandya district as well as from Livestock Research and Information Centre (Sheep), KVAFSU, Nagamangala. Carcass and meat quality characteristics were studied in 18 ram lambs belonging to three calpastatin genotypes (six animals per genotype).

Genetic Polymorphism of Calpastatin

About five ml of blood sample was collected from each animal in a vacutainer tube containing 0.5 per cent Ethylene Diamine Tetra Acetic acid (EDTA). The blood samples were immediately transported to the laboratory at 4 °C and genomic DNA was isolated within 24 hours by following high salt method as described by Miller *et al.* (1988) with necessary modifications. The quality and quantity of the genomic DNA were ascertained by UV spectrophotometer (Biophotometer plus, Eppendorf, Germany). Published primers viz., Forward: 5' TGGGGCCCAATGACGCCATCGATG 3' and Reverse: 5' GGTGGAGCAGCACTTCTGATCACC 3' (Palmer *et al.*, 1998; Nassiry *et al.*, 2006) were employed for amplification of exon 1C/ 1D and the intervening intron between them from a portion of the first repetitive domain of the ovine calpastatin gene.

The amplification reactions were carried out in 0.2 ml microfuge tubes using a Master cycler gradient (Bio Rad S1000, USA). The 25 µl reaction mixture consisted of 12.5 µl of Red PCR Master Mix (Amnion biosciences Pvt. Ltd), 1 µl (10 pmol/ µl) each of Forward (Exon 1C) and Reverse (Exon 1D) primer, 9.5 µl of PCR grade water and 1 µl (100 ng) of template DNA. The PCR protocol used for amplification involved an initial denaturation temperature of 95 °C (5 min), 33 cycles of 94 °C (1 min), 60 °C (1 min) and 72 °C (2 min), followed by final extension at 72 °C (8 min). Amplified PCR products were resolved on 1.5 per cent agarose gel along with 100 bp DNA ladder at a constant voltage of 100 V for 45 to 60 minutes in 1X TAE buffer. The restriction enzymes *MspI* (*Morexella* species) and *NcoI* (*Nocardia corallina*) were used to digest the PCR amplicons of calpastatin. The optimized RE digestion mixture consisted of 3.5 µl of autoclaved triple distilled water, 1 µl of 10 X assay buffer for RE, 0.5 µl of restriction enzyme (10 U/µl) and 5 µl of PCR product to form 10 µl digestion mixture. The reaction mixture was vortexed for few seconds for uniform mixing and then incubated at 37 °C for 12 hrs. The restriction enzyme digested PCR products were electrophoresed on two per cent agarose gel containing ethidium bromide (1%) along with 100 bp DNA ladder at a constant voltage of 100 V for 90 minutes using 1X TAE buffer. The restriction pattern resolved by agarose gel electrophoresis was photographed and analyzed using gel documentation system (Bio Rad, USA).

The genotype was determined by scoring the bands under the gel documentation system. The allele number, allele frequency and genotype frequency were calculated as described by Rosner (2005).

Carcass and Meat Quality Study

All the lambs selected were weaned at the age of three months and then raised under similar environmental conditions. Animals were fed a ration without limitation to achieve finishing weight. The animals were slaughtered after obtaining due permission from the Institutional Animal Ethics Committee (No. LPM/IAEC/172/2013, dt: 25.09.2013).

All the lambs were slaughtered at the age of six months with a 12 hours fasting period before slaughter. Pre slaughter weights were recorded and the animals were slaughtered under hygienic conditions by Halal method. Sticking, legging, dressing and evisceration were performed as per the procedure described by Gerrard (1964). The cut surface of Longissimus thoracis et lumborum muscle at the interface of 12th and 13th ribs on both side of the carcass was marked on tracing paper and measured by a planimeter (PLACOM Fuji, Japan) with standard procedure as loin eye area (cm²). The carcass was cut into different primal cuts viz., leg, loin, rack, neck and shoulder, flank, and breast and fore shank as per specifications of ISI (1995). Based on the pre slaughter weight dressing percentage was estimated. The assessment of pH of the Longissimus thoracis et lumborum muscle (2-4 hours after slaughter) was done using a digital pH meter. The meat color (Longissimus thoracis et lumborum muscle) was tested using Hunter Lab Mini scan XE plus separate colorimeter with geometry of diffuse/ 80 (sphere–8mm view) and an illuminant of D65/ 10 deg (Reston Virginia, USA). Colorimetry measures colour with quantitative physical methods and defines them within well-established numerical values. Here they are expressed using the standard Hunter L*, a*, b* system. The hue (relative position of colour between redness and yellowness) and chroma (colour intensity) was calculated as follows:

$$\text{Hue} = \tan^{-1}(b^*/a^*)$$

$$\text{Chroma} = \sqrt{(a^*)^2 + (b^*)^2}$$

Average value for each colour parameter was determined by taking observations from five different places of meat samples.

Water holding capacity was measured by the standard filter paper press method recommended by Grau and Hamm (1953 and 1957) with few modifications. The water holding capacity (%) was calculated by following formula:

$$\text{Water Holding Capacity (\%)} = \frac{\text{Area of compressed meat in cm}^2}{\text{Area of pressed out water in cm}^2} \times 100$$

The meat tenderness of Longissimus thoracis et lumborum muscle was determined by Warner-Bratzler shear (G-R Elec Co., Manhattan, USA) and expressed as kg/ cm². Meat chunks were sealed in low density polyethylene bags and placed in a water bath maintained at 80 °C for 30 minutes with final internal temperature of 60 °C. Later the temperature of meat chunks was brought to room temperature. The weight of meat chunks was recorded before and after cooking. The cooking loss was calculated by following formula and expressed in percentage.

$$\text{Cooking Loss (\%)} = \frac{\text{weight before cooking} - \text{weight after cooking}}{\text{weight before cooking}} \times 100$$



About 250 g of meat samples containing representative samples from all primal cuts were thoroughly minced, packed in polythene bags, labelled and kept at -80°C . Eighteen meat samples (Six samples for each CAST genotype) were sent to Animal Feed Analytical and Quality Assurance Laboratory, TANUVAS, Veterinary College and Research Institute, Namakkal, Tamil Nadu for proximate analysis with gross energy and omega 3 Fatty acids estimation. Descriptive statistics of different carcass weights, carcass components and meat quality traits were determined as per Snedecor and Cochran procedures (1989).

Association Study

By following ANOVA in GLM procedures of SAS 9.3(SAS Institute Inc., Cary, NC, USA), association between CAST genotypes and carcass and meat quality traits was analysed, keeping genotype as independent variables in linear model.

Results and Discussion

Genetic Polymorphism of Calpastatin

A 620 bp amplified product was resolved on 1.5 per cent agarose gel electrophoresis as shown in Fig. 1.

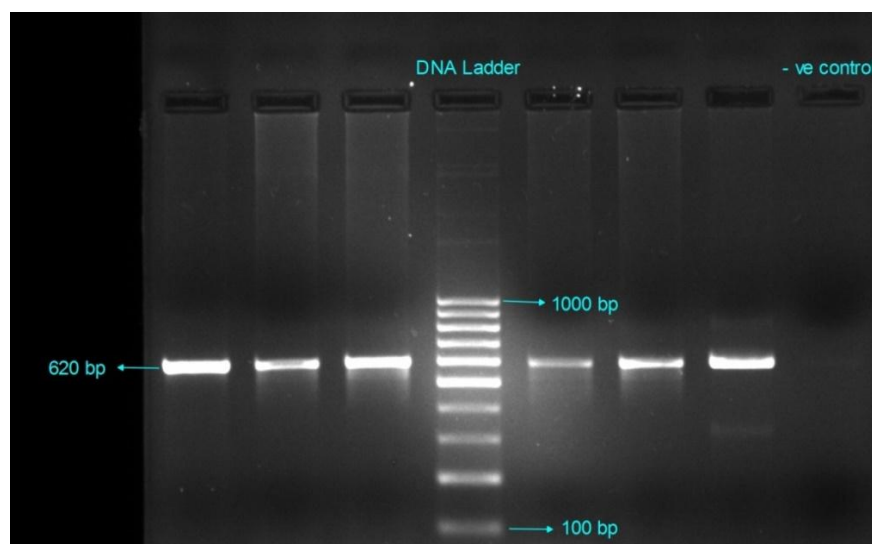


Fig. 1: Agarose (1.5 %) electrophoresis gel showing amplification of exon 1C/ 1D and the intervening intron region of calpastatin gene

The product size and identity of PCR products were further confirmed through sequencing of PCR products. Several studies reported amplification of ovine CAST gene (Szkudlarek-Kowalczyk *et al.*, 2011; Suleman *et al.*, 2012; Nikmard *et al.*, 2012) using same set of primers as used in the present study. RFLP digestion of PCR products yielded two alleles viz., M and N. The presence of these two alleles in CAST gene was well differentiated using the two restriction enzymes having differing restriction sites.

The PCR-RFLP pattern for animals that were homozygous for M allele (MM) produced two and one bands by *MspI* and *NcoI*, respectively, animals that were homozygous for N allele (NN) produced one and two bands by *MspI* and *NcoI*, respectively and the animals which were heterozygous (MN) yielded three bands each for both *MspI* and *NcoI* on two per cent agarose gel electrophoresis as shown in Fig. 2.

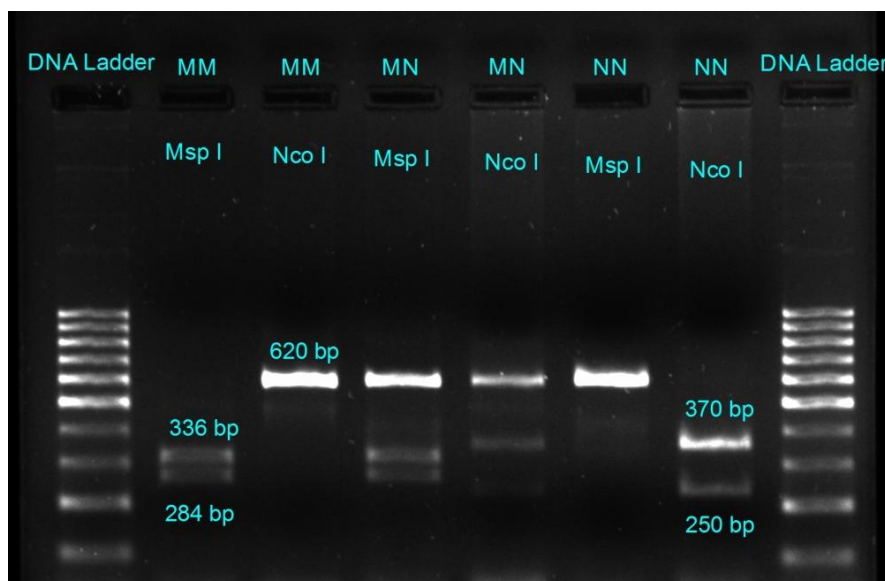


Fig. 2: Agarose (2 %) electrophoresis gel showing PCR-RFLP pattern of calpastatin gene
Lane 1 & 8 – 100 bp Ladder Lane 2, 4, 6 – CAST/*MspI* genotypes MM, MN & NN and
Lane 3, 5, 7 – CAST/*NcoI* genotypes MM, MN & NN

The allele and genotype frequencies and observed and expected heterozygosities for CAST gene observed in the Bandur ram lamb population are presented in Table 1. Further, the studied population was found to be in Hardy Weinberg equilibrium.

Table 1: Allele and genotypic frequencies, observed and expected heterozygosity and χ^2 value for CAST genotype

No. of Samples	Allele frequency		Genotypic frequency			Observed Hetero-zygosity Ho	Observed Hetero-zygosity He	Chi square value
	M	N	MM	MN	NN			
100	0.535	0.465	0.24 (24)	0.59 (59)	0.17 (17)	0.59	0.498	3.45 ^{NS}

Figures in the parenthesis showing number of animals

Earlier reports have confirmed that the restriction enzymes *MspI* and *NcoI*, by having different restriction sites, differentiated the two alleles present at CAST loci (Palmer *et al.*, 1998; Sutikno *et al.*, 2011; Szkudlarek-Kowalczyk *et al.*, 2011; Dehnavi *et al.*, 2012) in different breeds of sheep. The results are in concurrence with the reports of several workers in different sheep breeds viz., allele frequencies of 0.5545

and 0.4455 in Dalagh sheep of Iran (Azari *et al.*, 2012), 0.638 and 0.362 in Lori sheep (Nanekarani *et al.*, 2011), 0.544 and 0.456 in synthetic Karya sheep of Turkey (Ata and Cemal, 2013) were observed for M and N, respectively. Whereas, allele frequency of 0.69, 0.48 and 0.50 was observed for M allele in Ghezel, Arkhamerino and Ghezel x Arkhamerino sheep, respectively (Elyasi *et al.*, 2009).

In contrast to the present study, Palmer *et al.* (1998) reported 0.77 and 0.23 as the allele frequency for M and N alleles, respectively in Corriedale rams. Similarly, higher values for allele M were also reported in different sheep breeds of India (CSWRI, 2012), Iran (Shahroudi *et al.*, 2006 and Gharahveysi *et al.*, 2012), Slovakia (Gabor *et al.*, 2009), Pakistan (Suleman *et al.*, 2012) and Poland (Szkudlarek-Kowalczyk *et al.*, 2011). In agreement to the present study, Elyasi *et al.* (2009), Nanekarani *et al.* (2011), Azari *et al.* (2012) also reported higher MN genotype frequency than MM and NN in Arkhamerino and Ghezel x Arkhamerino sheep, Lori sheep and Dalagh sheep. Whereas, contrast to the present study, Palmer *et al.* (1998), Shahroudi *et al.* (2006), Gharahveysi *et al.* (2012) concluded that MM genotype was found to be higher in different sheep breeds. However, sheep breeds of Poland (Szkudlarek-Kowalczyk *et al.*, 2011) and Slovakia (Gabor *et al.*, 2009) showed only two genotypes, MM and MN.

With regard to the observed and expected heterozygosities, similar findings were reported by Ata and Cemal (2013) in Cine Capari (0.410 and 0.376) and Karya sheep (0.520 and 0.446). Whereas, low observed heterozygosity of 0.25 and 0.24 was reported in Zel (Dehnavi *et al.*, 2012) and Kurdi sheep (Nassiry *et al.*, 2007).

Association Study

Association between CAST Genotypes and Carcass Traits

The association between CAST genotypes and mean values of carcass weight and dressing percentage are presented in Table 2. The pre slaughter weight, hot carcass weight and dressing percentage were not significantly different with regard to CAST genotypes.

Table 2: Association between CAST genotypes and mean values of carcass weight and dressing percentage in Bandur ram lambs

S. No.	Particulars	CAST genotypes			
		MM	MN	NN	
1	Pre slaughter weight in kg	13.83 ± 0.42	14.02 ± 0.34	12.63 ± 0.41	NS
2	Hot carcass weight in kg	6.70 ± 0.25	6.53 ± 0.26	6.08 ± 0.17	NS
3	Dressing Percentage on PSW	48.41 ± 0.71	46.59 ± 1.37	48.30 ± 1.47	NS

NS: non significant

The association between CAST genotypes and non-carcass components, edible and inedible organ percentage and loin eye area are presented in Table 3. Among all the non-carcass components, only the skin weight of MM and MN genotype was significantly ($P < 0.05$) higher than NN genotype.

Table 3: Association between CAST genotypes and mean values of non-carass components, percentage of edible and inedible offal and loin eye area Bandur ram lambs

S. No.	Carcass Components	CAST genotypes			
		MM (g)	MN (g)	NN (g)	
1	Blood	363.70 ± 17.77	382.70 ± 15.08	338.70 ± 24.39	NS
2	Head	1037.00 ± 24.97	1043.00 ± 43.39	973.80 ± 32.10	NS
3	Foreleg	194.20 ± 4.89	200.80 ± 9.90	181.00 ± 8.03	NS
4	Hind leg	161.00 ± 4.26	169.20 ± 6.35	151.50 ± 6.33	NS
5	Skin	1118 ± 32.42 ^{ab}	1177 ± 30.80 ^a	1013 ± 42.47 ^b	*
6	Omental fat	271.70 ± 32.14	290.50 ± 30.46	224.70 ± 39.13	NS
7	Intestine	439.80 ± 16.37	454.30 ± 26.22	419.50 ± 19.59	NS
8	Stomach	468.30 ± 23.30	475.80 ± 26.72	445.00 ± 20.78	NS
9	Lungs & trachea	194.30 ± 17.35	209.20 ± 16.78	173.00 ± 10.25	NS
10	Total inedible offal	1466.00 ± 51.74	1522.00 ± 57.69	1376.00 ± 55.36	NS
11	Liver	257.00 ± 12.57	263.70 ± 20.33	253.80 ± 8.43	NS
12	Spleen	36.17 ± 5.39	35.83 ± 4.01	28.83 ± 2.99	NS
13	Heart	83.00 ± 4.24	82.00 ± 9.04	73.50 ± 3.96	NS
14	Kidney	42.50 ± 1.48	41.33 ± 2.23	38.50 ± 1.12	NS
15	Kidney fat	96.67 ± 12.29	97.17 ± 16.15	71.00 ± 10.16	NS
16	Testes	50.17 ± 10.55	44.00 ± 14.88	31.17 ± 11.87	NS
17	Total edible offal	565.50 ± 25.93	564.00 ± 32.65	520.20 ± 19.18	NS
18	Edible offal % PSW	4.08 ± 0.09	4.02 ± 0.17	4.01 ± 0.08	NS
20	Edible offal % HCW	8.43 ± 0.19	8.63 ± 0.31	8.49 ± 0.34	NS
21	Inedible offal % PSW	10.6 ± 0.23	10.86 ± 0.26	10.91 ± 0.35	NS
23	Inedible offal % HCW	21.93 ± 0.59	23.37 ± 0.77	22.74 ± 1.22	NS
24	Loin Eye Area (cm ²)	15.88 ± 1.31	15.85 ± 1.25	14.56 ± 0.83	NS

Means bearing same superscripts do not differ significantly; *Significant ($P < 0.05$), NS: non significant

The association between the CAST genotype and carcass primal cuts, and meat and bone composition of cuts are presented in Table 4. There was no significant difference between the carcass primal cuts, meat and bone composition and CAST genotypes. With regard to carcass traits, in the present study no significant differences were observed between the different CAST genotypes except skin weight.

In agreement to the present study, no significant association was observed between CAST genotypes (MM, MN, NN) with carcass traits (back fat thickness and longissimus muscle area post finishing) in Afshari sheep of Iran (Nikmard *et al.*, 2012). However, In a small groups of Dorset Down and Dorset Down x Coopworth lambs, 'AC' genotype was found to be associated with age corrected carcass weight (Palmer *et al.*, 1999a). Further, Kocwin and Kuryl (2003) reported that the animals with CAST genotype (PCR-RFLP) 'AA' had significantly higher ham weight and weight of the loin muscle in Landrace pigs.

Table 4: Association between CAST genotypes and mean values of carcass primal cuts and meat and bone composition of cuts in Bandur ram lambs

S. No.	Cuts	CAST genotypes			
		MM (g)	MN (g)	NN (g)	
1	Chuck				
	Meat	1555.00 ± 57.71	1536.00 ± 93.67	1396.00 ± 60.73	NS
	Bone	574.30 ± 25.21	529.30 ± 34.79	519.30 ± 40.50	NS
	Total	2129.00 ± 54.90	2065.00 ± 102.20	1915.00 ± 67.65	NS
2	Breast and Fore shank				
	Meat	408.70 ± 21.83	386.30 ± 23.15	366.00 ± 14.84	NS
	Bone	252.30 ± 31.84	186.7 ± 28.07	203.00 ± 21.55	NS
	Total	661.00 ± 50.18	573.00 ± 45.22	569.00 ± 30.10	NS
3	Rack				
	Meat	392.30 ± 40.34	407.30 ± 45.98	378.70 ± 12.96	NS
	Bone	264.30 ± 28.23	242.70 ± 27.70	255.00 ± 17.26	NS
	Total	656.70 ± 64.33	650.00 ± 61.74	633.70 ± 21.59	NS
4	Flank	250.30 ± 36.00	301.30 ± 23.35	219.00 ± 29.97	NS
5	Loin				
	Meat	519.70 ± 55.71	477.70 ± 43.10	441.00 ± 52.98	NS
	Bone	202.00 ± 18.95	187.30 ± 10.91	199.70 ± 15.95	NS
	Total	721.70 ± 47.50	665.00 ± 48.09	640.70 ± 59.66	NS
6	Leg				
	Meat	1697.00 ± 63.82	1653.00 ± 75.99	1492.00 ± 52.70	NS
	Bone	438.00 ± 56.94	487.00 ± 22.47	502.00 ± 12.00	NS
	Total	2135.00 ± 75.40	2140.00 ± 84.31	1994.00 ± 60.27	NS
7	Fore quarter	3447.00 ± 140.20	3288.00 ± 152.10	3118.00 ± 90.80	NS
8	Hind quarter	3107.00 ± 109.80	3106.00 ± 123.10	2854.00 ± 103.40	NS

NS: non significant

Association between CAST Genotypes and Meat Quality Traits

The association between the CAST genotypes and meat quality traits are presented in Table 5.

Table 5: Association between CAST genotypes and mean values of meat quality traits in Bandur ram lambs

S. No.	Traits	CAST genotypes			
		MM	MN	NN	
1	pH	6.27 ± 0.03	6.38 ± 0.07	6.41 ± 0.12	NS
2	Shear force (kg/ cm ²)	3.968 ± 0.19 ^a	4.185 ± 0.12 ^a	3.09 ± 0.13 ^b	***
3	Cooking loss %	28.78 ± 4.24	26.84 ± 3.67	22.42 ± 3.61	NS
4	WHC %	46.90 ± 3.19	44.34 ± 6.06	57.98 ± 6.70	NS
5	Back Fat Thickness (cms)	0.25 ± 0.03	0.27 ± 0.03	0.27 ± 0.02	NS
6	Hunter color Score				
	L*	52.31 ± 0.88	52.61 ± 1.25	51.16 ± 1.24	NS
	a*	12.31 ± 0.38	11.66 ± 0.16	11.85 ± 0.23	NS
	b*	13.28 ± 0.81	12.62 ± 0.42	13.03 ± 0.61	NS
	Hue	46.95 ± 1.76	47.19 ± 0.68	47.55 ± 1.34	NS
	Chroma	18.14 ± 0.71	17.19 ± 0.40	17.64 ± 0.51	NS

Means bearing same superscripts do not differ significantly; *** Significant (P<0.001), NS: non significant

The Warner-Bratzler shear force value of NN genotype was significantly ($P < 0.001$) lower than MM and NN genotype indicated more tenderness of meat. There was no significant difference between meat quality traits such as pH, WHC, cooking loss, back fat thickness and Hunter color score, and CAST genotypes. Similar to the present study, ewes genotyped as 'AC' had significantly higher ($P < 0.05$) shear force measurement than 'AA' or 'AB' (Palmer *et al.*, 1999b) and No significant association between the CAST genotypes and meat quality traits viz., pH, tenderness, water holding capacity and cooking loss was observed in local thin tail sheep (Dagong *et al.*, 2012).

Supporting to the present study, Schenkel *et al.* (2006) reported that the animals with 'CC' genotype yielded beef that was more tender and had greater fat yield ($1.44 \pm 0.56\%$) ($P < 0.05$) than GG genotype.

The association of CAST genotypes with proximate principles and fatty acid profile are presented in Table 6.

Table 6: Association between CAST genotypes and mean values of proximate principles and fatty acid profile in Bandur ram lambs

S. No.	Parameters	CAST genotypes			
		MM	MN	NN	
A. Proximate Analysis					
1	Moisture %	72.11 ± 0.68	72.07 ± 0.67	72.13 ± 0.77	NS
2	Crude protein %	19.20 ± 0.72	20.43 ± 0.50	19.66 ± 0.54	NS
3	Crude fibre %	0.24 ± 0.06	0.24 ± 0.05	0.24 ± 0.04	NS
4	Ether extract %	7.33 ± 0.84	7.21 ± 0.87	7.14 ± 0.91	NS
5	Total ash %	0.99 ± 0.05 ^{ab}	0.88 ± 0.07 ^a	1.16 ± 0.07 ^b	*
6	Gross Energy (Kcal/ kg)	1799.00 ± 63.79	1847.00 ± 74.97	1806.00 ± 67.98	NS
B. Fatty acid profile					
	Saturated Fatty acids				
1	Myristic acid %	4.58 ± 0.24	4.17 ± 0.54	3.71 ± 0.74	NS
2	Palmitic acid %	27.06 ± 0.69	26.65 ± 0.97	27.96 ± 1.26	NS
3	Stearic acid %	16.70 ± 0.98	17.16 ± 1.22	16.97 ± 2.07	NS
4	Arachidic acid %	0.23 ± 0.04	0.26 ± 0.02	0.26 ± 0.09	NS
5	Behenic acid %	1.08 ± 0.29	1.19 ± 0.25	0.93 ± 0.23	NS
	Unsaturated Fatty acids				
6	Palmitoleic acid %	2.56 ± 0.56	2.59 ± 0.51	2.92 ± 0.86	NS
7	Oleic acid %	40.66 ± 1.35	41.58 ± 1.50	40.33 ± 1.19	NS
8	Linoleic acid %	5.30 ± 0.56	5.01 ± 0.64	5.30 ± 0.70	NS
	Omega 3 Fatty acids				
9	Linolenic acid %	0.25 ± 0.06	0.22 ± 0.05	0.32 ± 0.08	NS
10	Ecosapentaenoic acid %	0.26 ± 0.04	0.33 ± 0.07	0.18 ± 0.04	NS
11	Docosahexaenoic acid %	0.24 ± 0.03	0.21 ± 0.02	0.19 ± 0.02	NS
12	Others %	1.08 ± 0.11	0.64 ± 0.13	0.90 ± 0.22	NS

Means bearing same superscripts do not differ significantly; *Significant ($P < 0.05$), NS: non significant

The ash content was significantly higher ($P < 0.05$) in NN genotype. There was no significant difference between the other proximate principles analysis parameter or fatty acid profile and CAST genotypes. There are no earlier reports on association between CAST genotypes and proximate principles and fatty acid profile in Bandur and other sheep breeds for comparison.



In nutshell, this study is the first report on the association of CAST genotypes with carcass and meat quality traits in Bandur lambs. The genetic variability in calpastatin loci was successfully determined by PCR-RFLP in Bandur sheep. High loin eye area recorded indicated higher muscular development in Bandur ram lambs. The lambs genotyped as NN had more tender meat than MM and NN genotyped lambs.

Conclusion

It may be concluded that CAST gene has potential effect on meat tenderness and may be considered as candidate gene to improve meat tenderness in sheep, especially in Bandur sheep. But, further confirmation is warranted involving larger population size.

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