Effects of polymorphisms in CAPN1 gene on meat tenderness in southern and Crossescattle

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Abstract

This study aimed to detect single nucleotide polymorphisms (SNPs) of the CAPN1 gene in cattle using 139 animals of the southern and crossbreed cattle breeds, 73 and 66 calves of the two breeds, respectively, and to know the relationship of these SNPs in meat characteristics specific to tenderness. Blood and meat samples were collected and tested. The technique of polymerase chain reaction (PCR) was used, and the technique of DNA sequencing was used to detect the genetic structures of the gene. The frequency of the genotypes of the CAPN1 gene (GG), CG and (CC) was 85.00, 6.80 and 8.20%, respectively, in the southern cattle breed, and 56.00, 32.00 and 12.00% in the breed of crossed cows at site 316, and the genotypes AG and GG were (68.00 and 32.00)% for the southern and (26.00 and 74.00)% for the racket, respectively, at site 530. As for the allelic frequency of the alleles, the percentages ranged from low to high. Significant differences appeared (P<0.05)) according to the genotypes of the studied traits, as the two structures outperformed The genotypes GG and AG in most of the traits and CG in some traits in the current study, which included the concentration of myoglobin pigment, the amount of metamyoglobin, the fiber breakdown index, total and soluble collagen and myofibrillar proteins.

Keywords;- polymorphism , CAPN, tenderness , Crosses , cattle
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Introduction

Single nucleotide polymorphisms (SNPs) are the most efficient molecular markers (Faraj et al., 2020) and molecular markers offer new opportunities to accelerate selection, and the ability to increase genetic improvement rates using genetic information will, in the first step, be mainly limited to important species and strains. Local or global within species (Erhardt and Weimann, 2020). The discovery of DNA markers associated with meat quality traits has become a topic of interest in cattle breeding. The selection of CAPN1 as a candidate gene for the quantification of repeat single nucleotide polymorphisms (SNPs) associated with meat quality traits in cattle is a major contributor to future cattle breeding. (Sun et al., 2018) Genes that have an important role in meat quality are calcium-activated neutrophil protease genes, known as calpains (CAPN) (Dairoh et al.,2021). The CAPN1 gene is an important indicative of meat tenderness in cattle (Xin et al., 2010). The CAPN1 system is responsible for the degradation of myofibril proteins and the tenderness of meat after slaughter (Lee et al., 2019).

Materials and working methods

The study was carried out in the laboratories of the College of Agriculture - University of Basra for the period from 11/14/2020 to 02/10/2021,

drawing 5 ml of blood from the jugular vein from each animal before slaughter, and meat samples were taken from the dorsal region. The genetic material DNA was extracted from blood in the Molecular Genetics Laboratory for the purpose of determining the genetic structures. Chemical tests were conducted in the Physiology Laboratory. Physical and sensory tests were also conducted on meat samples in the Meat Science Laboratory to identify samples with good traits to identify the genetic variants with the desired traits.

Collection of blood samples and DNA extraction

3 ml of blood from each animal was placed in test tubes containing EDTA K3 anticoagulant, transferred to a cooler box and kept at -18 °C until laboratory analysis was performed.

The DNA was extracted using the kit of the Korean company Genaid, according to the manufacturer's steps, and the following steps were taken:

1- (200) microliters of blood were withdrawn via a micropipe and placed in a 1.5 ml Oppendorf tube.

2- (200) microliters of PBS buffer solution were added to the Alpendorf tube.

3- (20) µl of Proteinase K solution was added to it and mixed by pipette.

4- (200) µl of GSB cell membrane breaking solution was added, then samples were shaken with vortex for 10 seconds, and incubated at 60° C. for 5 minutes with the tube turning over every two minutes.

5- Put (200) microliters of concentrated ethanol alcohol (100%), then shake with Vortex for 10 seconds, then empty the contents into a filter (GS column) and use centrifugation at a speed of (15000 rpm) for one minute, and then dispose of filtrate

6- (400) microliters of the first washing solution (W1-Wash buffer) were added, and centrifugation was used at a speed of (15,000 rpm) for a period of (30 seconds), after which the filtrate was disposed of.

7- (600) microliters of the second washing solution (Wash Buffer) were added, and centrifugation was used at a speed of (15000 rpm) for (30) seconds and the filtrate was disposed of.

8- The filter tube was placed in a new (1.5) ml Appendorf tube.

9- (100) microliters of (Elution buffer) solution (which was pre-heated) was added at 50-60°C and centrifuged at 15000 rpm for (30) seconds, then new DNA was collected and sealed and kept by freezing at 20°C. -)) until you make the other steps.

A segment of the CAPN1 gene was PCR amplified using primers published in GenBank under number AF248054 and according to the following sequence.

5'-AGCAGCCCACCATCAGAGAAA – 3' F

5'- TCAGCTGGTTCGGCAGAT – 3' R

Myoglobin concentration

The myoglobin concentration was measured according to the method of Zessin et al (1961), and the myoglobin concentration was calculated according to the following equation:

Myoglobin concentration = (Absorbance x 2.4)/(Model weight x 0.452)**Metmyoglobin**

I followed the method (1998). Lee et al mentioned in (Sadkhan and Al-Moussawi) (Sadkhan and Al-Moussawi) for the determination of Metmyoglobin pigment The percentage of Metmyoglobin pigment was calculated based on the following equation:

$$\textbf{(Met-Mb)\%=1.39-}\frac{A700-A572}{A700-A525}\times 100$$

Muscle Fibers Index

MFI was calculated according to the method mentioned by Jeremiah and Martin, (1982), calculated by the following equation. Muscle fiber breakage index = weight of the precipitate (gm) ×100

The solubility of myofibrillar proteins

The method mentioned by Talmant and Ouali (1990) was used to estimate the solubility of

myofibrils proteins and to estimate the concentration of myofibers proteins according to Bayuret method described by Gornall et al (1949). Solubility was expressed as mg protein/g meat. Model Weight (gm)

Measurement of soluble and insoluble collagen content

The method of et al. (1991 (Morgan et al., 1991) was used to estimate the soluble collagen content.

estimation of collagen

To get the true value of the collagen content in both the filtrate and the precipitate, the value of the hydroxyproline concentration obtained in the filtrate is multiplied by (7.52) and the concentration of hydroxyproline acid obtained in the precipitate at (7.25) based on what was indicated by Cross *et al*., (1973).

Statistical analysis

Use the ready-made statistical program (2020) SPSS, to find the significant differences between the means.

Results and discussion

DNA Extraction

The process of DNA extraction, measurement of concentration, purity and detection is an initial step for amplification and detection of genotypes, as extraction was done using the Kit)) supplied by Geneaid Company according to the manufacturer's steps, and then the concentration and purity of DNA (ng/µl) for each sample was measured by a device Nano drop, and then the DNA samples were removed with agarose gel. It is clear from Figure (1) that the extraction process was successful as a first step to study the CAPN1 gene.

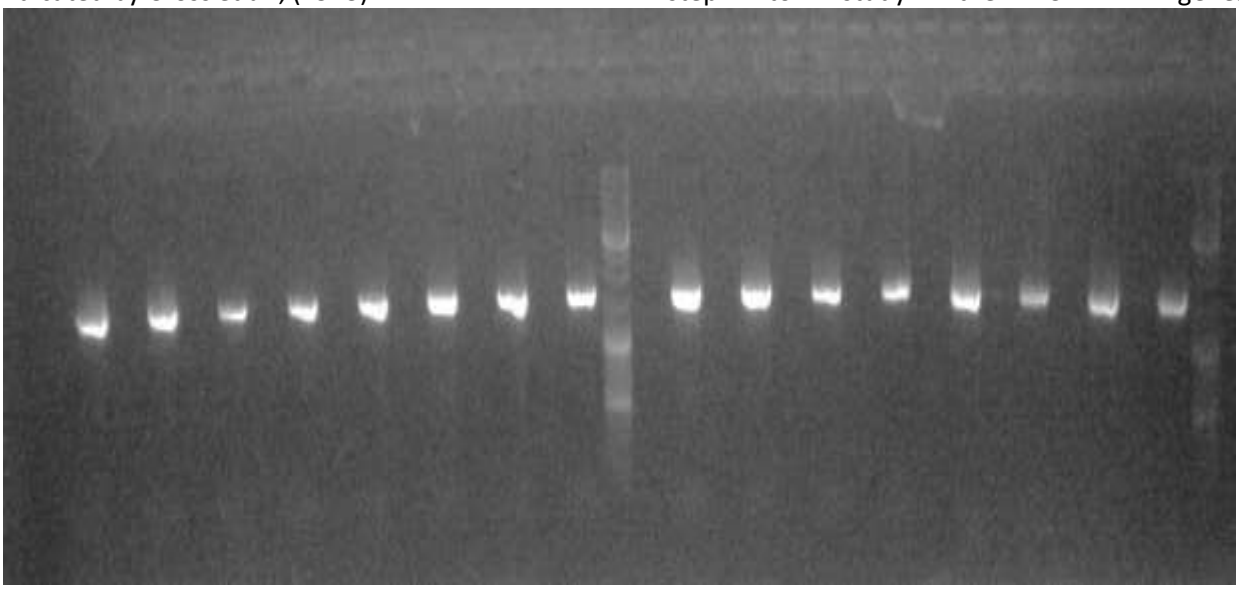

Figure (1) Electrophoresis results of the extracted DNA samples

Table (1) shows the ratios of allelic and genetic frequencies. One hundred and thirty-nine genetically bulls were classified for two different markers on the CAPN1 gene. Of the southern and club bulls of 73 southern bulls and 66 club bulls, the results of the analysis showed nocturnal frequencies with moderate to high proportions of alleles that are related to softness in each CAPN1 name. The frequency of the secondary alleles (C) was 0.12 for the southern, 0.28 for the striker, (G) 0.16 for the southern, and 0.13 for the hitter for the two loci SNP 316 and SNP 530, respectively. The high frequencies were (G) 0.87 for the southern and 0.72 for the hitter and (A) 0.84 for the southern and 0 .87 of the racket for the SNP 316 and SNP 530 loci, respectively. There are small percentages (0.082% and 0.12%) having the CC genotype for the southerner and the striker, respectively, in SNP 316. The GG genotype in SNP 530 was the least frequent, where the frequency of the gene was 0.32% and 0.26% for

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the southern and the striker, respectively, in While no homozygous AA bulls were found in the entire sample. The majority of SNPs were found in introns or were synonymous substitutions, except for one substitution in exon 9 (C<G) and another in exon 14 (G<A) (SNP 316 and SNP 530, respectively). SNP 316 (C/G alleles) specifies the substitution of the histidine Ala to Gly at 316 for the protein and the other (G/A alleles) causes the change of Val to Ile at position 530.

Table (1) The genotype and allelic repeats of the CAPN1 gene locus of the southern and Crosses cattle breeds

Breed		**CAPN1**	**316**			**CAPN1**	**530**		
	CC	**CG**	**GG**	**C**	**G**	**AG**	**GG**	**A**	G
Jenoubi(n=73)	0.082	0.068	0.85	0.13	0.87	0.68	0.32	0.84	0.16
Crosses(n=66)	0.12	0.32	0.56	0.28	0.72	0.74	0.26	0.87	0.13

Effect of ancestry and polygenic phenotypes of the calpain gene on myoglobin pigmentation

Table (2) shows the effect of the calpain gene genotypes on the concentration of myoglobin pigment mg/g of meat. The results recorded a significant superiority (P<0.05) for the genotype GG and CC, whose values were 5.58 and 5.20 mg/g of meat, respectively, on the genotype CG, which had a value of 4.06 mg. \g of meat in myoglobin pigment at site 316 in the southern strain, as well as the superiority of the AG genotype, which amounted to 5.58 mg/g of meat, over the genotype GG, which amounted to 4.85 mg/g of meat for the same strain at site 530 (Table 3), while in the strain The results of Table (2) showed that the CG genotype of 4.53 was superior to the GG genotype of 4.09 mg/g of meat at site 316, and the genotypes GG and AG did not differ significantly from each other at site 530 Table (3). This difference may be due to The myoglobin dye leads to the difference in the genetic structures of individuals, as well as the treatment of the animal before slaughter has a role in the color of the meat, as a gentle treatment leads to a decrease in the consumption of oxygen, and thus increases the oxymyoglobin, and this in turn shows the bright red color**.**

Effect of strain and polygenic phenotypes of the calpain gene on metamyclopene pigment

We note from Table (2) that there is a significant effect (P<0.05) for the genotypes of the calpaine gene, as the genotypes differed within the breed and for both breeds. 28.25 % and composition CG with a value of 23.12 % for the southern strain at site 316. As for the striking strain, the results in Table (3) showed a significant superiority of the genotype GG over the other structures, which recorded the lowest value of the percentage of metamiclobin pigment, which amounted to 26.79%, which is considered the best result. While the value of the CG genotype was 29.79%, and the CC genotype amounted to 34.13%, although all the genotypes differed among themselves at site 316. While the results in Table (3) did not show any significant effect of the genotypes of methamiclobin for both strains in Location 530.

Effect of strain and polymorphism of the calpain gene on the evidence of fiber breakage

Table (2) indicates that there is a significant effect (P<0.05) of the calpain gene genotypes on the fiber breakage index of the southern strain. 316, while the results in Table (3) did not show any significant effect of the genotypes of the calpaine gene on the fiber breakage index of the southern strain at site 530, and the

results did not show any significant effect of the genotypes on the fiber breakage index of the striking strain at site 316 and 530.

Table (2) Effect of strain and genotype of CAPN1 gene on meat chemical traits (myoglobin stain,

Breed	Trait / Marker	Myoglobin concentration	Rate Metmyoglobin	Muscle Fibers Index
	CAPN316			
Jenoubi	GG	a5.58 ±0.16	b28.25 ±0.76	b5.44 ± 0.10
	CG	b4.06 ±0.37	c23.12 ± 1.80	b5.61 ± 0.23
	CC	a5.20 ±0.70	a33.17 ±3 .40	a6.54 ± 0.57
cross	GG	b4.09 ±0.20	c26.79 ±0.96	5.19 ± 0.12
	CG	a4.53 ±0.21	b29.79 ± 1.04	5.18 ± 0.13
	CC	ab4.53 ±0.37	a34.13 ±1.80	5.14 ± 0.23

metamiclobin stain and fiber breakage index) SNP 316

•The averages in one column within the breed bearing different letters differ significantly at the 5% probability level.

Table (3) Effect of strain and genotype of CAPN1 gene on meat chemical traits (myoglobin stain,

Breed	Trait / Marker	Myoglobin concentration	Rate Metmyoglobin%	Muscle Fibers Index

metamiclobin stain and fiber breakage index) SNP 530

	CAPN530			
Jenoubi	GG	b4.85±0.26	28.39± 1.35	5.46± 0.15
	AG	a5.58± 0.18	27.22± 0.93	5.51± 0.10
cross	GG	4.25± 0.27	27.71± 1.40	5.00± 0.16
	AG	4.35± 0.16	29.42± 0.85	5.25± 0.09

•The averages in one column within the breed bearing different letters differ significantly at the 5% probability level.

Effect of strain and genotypes of the calpain gene on the amount of total collagen

Table (4) shows that there is a significant effect (P<0.05) of gene genotypes on the amount of total collagen in the Southern strain, as there was a significant superiority of genotype GG and CG, whose values were 4.51 and 4.40, respectively, on the CC genotype, which reached a value of 4.91 in the locus. 316, while in the striking strain, the results showed a significant superiority of the CC genotype, which amounted to 4.26, over the genotypes CG and GG, whose results were 4.45 and 4.52, respectively, at site 316.

The results of Table (5) did not show any significant effect of the genotypes on the total collagen quantity of the two strains at site 530. And there is an inverse relationship between the amount of collagen and the softness characteristic, meaning that the decrease in the amount of collagen leads to an increase in the softness characteristic.

Effect of strain and polygenic phenotypes of the calpain gene on the percentage of soluble collagen

Table (4) indicates that there was no significant effect of the calpain gene genotypes on the percentage of soluble collagen in both strains at site 316, while in site 530, Table (5), a significant difference (P<0.05) was found between the calpain gene genotypes at site 530. In the southern strain, the AG genotype, which amounted to 28.42, was superior to the GG genotype, which amounted to 27.69, and the results did not record any significant differences for the genotypes of the striking strain.

Effect of strain and polymorphism of the calpain gene on the solubility of myofibrillar proteins

The results of Table (4) indicate that there is a significant effect (P<0.05) of the calpain gene genotypes on the solubility of myofipril proteins mg protein/gm meat. While the two structures did not differ significantly from the CC genotype in the Southern strain. The results also indicated a significant superiority for the CC genotype, which amounted to 50.79 mg protein/gm meat, over the GG genotype, whose value was 49.48 mg protein/gm meat, in the striking strain at site 316. The results did not record any significant effect of the genotypes in Table (5). The calpain gene is located at site 530 of the southern and striker breeds, and the calpain system is the main contributor to the post-slaughter protein degradation that correlates with the tenderness of meat (Nowak, 2011). The increase in solubility may be attributed to the activity of the calpain enzyme, which contributes to the fragmentation and breakdown of z-line Saucedo et al., 2021) proteins**.**

Table (4) Effect of the strain and genotype of CAPN1 gene on meat chemical traits (total collagen, soluble collagen percentage and solubility of myofibrillar proteins) SNP 316

Breed	Trait / Marker	Total collagen	Collagensolubilized	The solubility of myofibrillar proteins
	CAPN316			
Jenoubi	GG	b4.51± 0.04	28.18± 0.26	a53.70± 0.23
	CG	b4.40± 0.09	27.95± 0.62	b52.64± 0.54
	CC	a4.91± 0.23	29.60± 1.51	ab52.61± 1.33
Cross	GG	a4.52± 0.05	27.79± 0.33	b49.48± 0.29
	CG	a4.45± 0.05	27.97± 0.36	ab50.05± 0.31
	CC	b4.26± 0.09	28.20± 0.62	a50.79± 0.54

•The averages in one column within the breed bearing different letters differ significantly at the 5% probability level.

Table (5) Effect of strain and genotype of CAPN1 gene on meat chemical traits (total collagen, soluble collagen percentage and solubility of myofibrillar proteins) SNP 530

Breed	Trait / Marker	Total collagen	Collagen solubilized%	The solubility of myofibrillar proteins
	CAPN530			
Jenoubi	GG	4.47± 0.06	b27.69±0.41	53.40± 0.38
	AG	4.52± 0.04	a28.42±0.28	53.57± 0.26
cross	GG	4.49± 0.06	28.04±0.42	49.84± 0.39
	AG	4.44± 0.04	27.87±0.25	49.90± 0.24

•The averages in one column within the breed bearing different letters differ significantly at the 5% probability level.

Conclusions

We conclude from this study that there is a close relationship between the genetic forms and the tenderness of meat, as a large discrepancy was found between the genetic forms of the CAPN1 gene in local and cross-bred cattle. And the use of molecular markers will contribute to shortening the time and effort in identifying animals with desired traits by the breeder.

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