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Article

Relationship between various meat qualities, tenderness, and Jenoubi and crossbreed cows' CAPN1 gene polymorphism

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Abstract
139 calves of the Jenoubi breed and crossbred were used (73 and 66 calves, respectively), their ages ranging between 2-3 years, from Thi- Qar governorate. Blood and meat samples were collected, and tests were performed on them. The polymerase chain reaction (PCR) and sequencing techniques were used to detect the gene genotypes. The results showed moderate to high allelic frequencies that correlate with the tenderness of the CAPN1 gene. The two genotypes, GG and AG, showed significant variations ($P< 0.05$) in most sensory traits at sites 316 and 530, including tenderness, juiciness, and general acceptability.
Additionally, the genotype GG within the Jenoubi breed significantly outperformed in pH, water holding capacity, and cooking loss, with values of 5.81, 7.65, and 49.76, respectively. While the GG genotype of cross animals at site 316 showed better water-holding capacity and cooking loss. There are relationships between genotypes of this gene in two different sites so that it might be used in the selection program of local and their crosses.
Keywords: CAPN1 gene, Meat quality, Tenderness, Jenoubi cattle.

Introduction

The application of markers that support selection, or Marker Assisted Selection (MAS), is crucial for identifying genetic variation in cattle populations since the genetic polymorphisms of the local animal genes are one of the fundamental factors supporting selection[1,2,3]. The genetic improvement of beef cattle in terms of improving quantitative and qualitative traits depends mainly on molecular markers[4]. Molecular markers are an aid in determining an animal's genetic makeup and thus predicting its performance[5]. Selection with this technique is a very important step in breeding programs[6]. Molecular marker information can be beneficial for identifying animals with high genetic merit for tenderness, and selection can be performed on younger, slaughtered and even prenatal animals for meat quality traits[4,7].
Meat quality is a significant economic factor influenced by numerous genes and the environment. In recent years, significant developments in molecular genetics have enabled the discovery of loci, or gene-related markers, that affect meat quality and their chromosomes[8]. One of these identified genes is the calpain CAPN1 gene, which plays a vital role in meat tenderness and indirectly acts on meat quality traits[9]. In comparison, the CAPN1 gene encodes the enzyme μ-calpain responsible for the degradation of myofibroblasts[10].

The present study aimed to determine the relationship of the genetic polymorphisms of the CAPN1 gene with tenderness and some other meat characteristics.

Materials and Methods

From November 14, 2020, to April 26, 2021, the experiment was conducted in the labs of the College of Agriculture/University of Basrah. Before the animals were slaughtered, blood samples were taken. Meat samples were obtained from the dorsal area following the slaughter. DNA was extracted from blood in the molecular genetics laboratory. Chemical tests were conducted in the physiology laboratory. Physical and sensory tests were also conducted on meat samples in meat science.

Value (pH) pH

The pH was estimated in meat samples based on the method mentioned by Verma et al.[11] by homogenizing 10 g of meat sample with 100 ml of distilled water using a German-made Gerhardt homogenizer. The mixture was filtered through filter paper No. 1.

Water Holding Capacity (WHC)

Meat water holding capacity was estimated according to the method mentioned by Al-Tai and Al-Moussawi[12] by weighing 10 g of the sample and adding 20 ml of distilled water. The sample was mixed well. Then, the contents were transferred to a graduated cylinder, at the end of which is a filter paper funnel. The filtrate volume was recorded after 30 minutes, and the water-holding capacity of the meat was calculated according to the following equation:

WHC (ml) = Total water volume (ml) - Amount of leachate (ml).

Cooking losing test

It was calculated by roasting the meat pieces in the electric oven at a temperature of 200 until the grilling was completed according to the following equation:

Cooking losing%= (weight before cooking- weight after cooking)*100/weight before cooking

Panel test

After the meat slices were cut in dimensions of 3 and grilled, a sensory evaluation was conducted by several experienced judges in the Animal Production Department to evaluate the samples in terms of color, tenderness and juiciness (All acceptability) according to a scale of 9 degrees[13].

Blood collection and DNA Extraction

Three 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. Genomic DNA was extracted from whole blood using the gSYNC™ DNA Extraction Kit manufactured by the Taiwanese Geneaid Company according to the manufacturer's steps. DNA extraction purity is conducted as the concentration and purity of DNA (ng/µl) for each sample were measured by a Nanodrop device. Then, the DNA samples were detected with agarose gel. A segment of the CAPN1 gene was PCR amplified using primers published in GenBank under No. AF248054 and according to the following sequence

F5'-AGCAGCCCACCATCAGAGAAA–3', R 5'-TCAGCTGGTTCGGCAGAT–3'

Statistical analysis

Using the statistical program SPSS (version 27, 2020)[14], ANOVA was utilized to compare study traits due to genotypes and breeds. The statistical differences between means were evaluated using the least significant test within the same statistical software.

Results

Extraction DNA

It is clear from Figure (1) that the extraction process was successful as a first step in studying the CAPN1 gene.

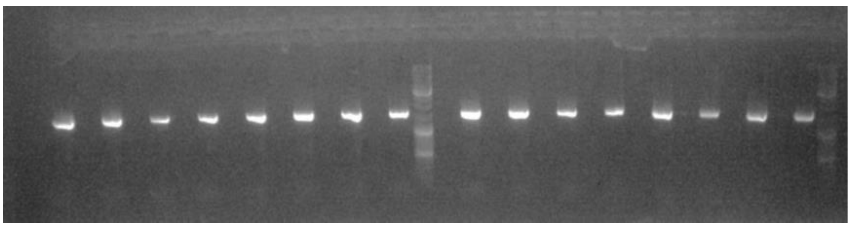

Figure (1) Electrophoresis results of the extracted DNA samples.

CAPN1 gene polymorphisms

Table 1 and 2 shows the proportions of allelic and genotype frequencies. One hundred and thirty-nine genetically modified calves were classified for two different markers on the CAPN1 gene at 316 and 530 pb regions. The frequency of allele C in the first region was 0.12 and 0.28, and G was 0.87 and 0.72 for Jenoubi calves, respectively. Those of G and A alleles in the 530 regions were 0.16, 0.13, 0.84 and 0.87, respectively. For Jenoubi and cross, the proportions of the CC genotype are negligible (0.082 and 0.12, respectively). The SNP 530 GG genotype had the lowest frequency, with genotype frequencies of 0.32 and 0.26 for Jenoubi and cross, respectively. For the entire sample, no homozygous AA calves were discovered. Except for two substitutions in exons 9 (CG) and 14 (GA), most of the SNPs were discovered in introns or were synonymous changes (SNP 316 and SNP 530, respectively). In SNP 316 (C/G alleles), one substitution Ala to be Gly and the other (G/A alleles) causes a change of Val to Ile at position 530.

Locus	Breed	Allele	Frequency	Locus	Breed	Allele	Frequency
316	Jenoubi	C	0.12	350	Jenoubi	G	0.16
		G	0.87			A	0.84
	Cross	C	0.28		Cross	G	0.13
		G	0.72			A	0.87

Table 1. Frequency of CAPN1 gene alleles in the studied sample of Jenoubi and cross calves.

Marker	Jenoubi	Cross	Total number

		No.	Frequency	No.	Frequency	(frequency)
316	GG	62	0.85	37	0.56	99 (0.71)
	CG	5	0.07	21	0.32	26 (0.19)
	CC	6	0.08	8	0.12	14 (0.10)
	Total	73	1.00	66	1.00	139 (1.00)
530	GG	23	0.32	17	0.26	40 (0.29)
	AG	50	0.68	49	0.74	99 (0.71)
	Total	73	1.00	66	1.00	139(1.00)

Table 2. Frequency of CAPN1 gene genotypes in the studied sample of Jenoubi and cross calves.

Effect of genetic polymorphisms of CAPN1 gene on physical characteristics of meat

pH

Table 3 indicates that there is a significant effect between the genotypes of the Calpine gene in the Jenoubi breed for the pH trait, as the GG genotype, with its value of 5.81, outperformed (P<0.05) the genotype CC (6.26). The CG genotype did not differ significantly from the GG genotype at site 316, which had a value of 5.88. The results did not show any significant differences between the genotypes of the Calpine gene for the cross calves at the same site. As for site 530, Table (3) indicates that there is also a significant effect of the genotype within the Jenoubi breed for the pH value, as the AG recorded the highest value (5.87) than the genotype GG (5.75). There was no significant effect on the genotypes of the striking strain.

Water holding capacity

The CC and GG genotypes of 316 site showed higher (P<0.05) water holding capacity (8.00 and 7.65 ml) than that of the CG genotype (5.55 ml) of the Jenoubi breed (Table 3). which did not differ significantly between them, were superior to the CG genotype, where their values were 8.00, 7.65 and 5.55 ml, respectively, in Southern Dynasty at location 316. Similar results were shown by the GG genotype of cross calves (site 316), which recorded higher (P<0.05) water-holding capacity than those of CC and CG genotypes. However, genotypes at 530 sites exhibited similar values of water-holding capacity in both Jenoubi and cross calves. As well as Jenoubi and cross calves recorded similar water-holding capacity means.

Breed	Genotypes	pH	WHC	Cooking loss
Marker 316				
Jenoubi	GG	5.81b±0.03	7.65a±0.17	49.76b±0.74
	CG	5.88b±0.08	5.55b±0.40	55.32a±1.74
	CC	6.26a±0.19	8.00a±0.18	46.58b±3.26
	p-value	0.05	0.05	0.05
	Mean	5.98A±0.07	7.07±0.36	50.55±1.55
cross	GG	5.80±0.04	7.78a±0.22	50.16b±0.93
	CG	5.81±0.05	7.09b±0.23	55.16a±1.00
	CC	5.75±0.08	7.15b±0.40	51.47b±1.74

	p-value	NS	0.05	0.05
	Mean	5.79B±0.03	7.34±0.17	52.26±0.74
Marker 530				
Jenoubi	AG	5.87a±0.03	7.35±0.21	50.12±0.91
	GG	5.75b±0.05	7.33±0.31	51.35±1.31
	p-value	0.05	NS	NS
	Mean	5.98A±0.07	7.07±0.36	50.55±1.55
cross	AG	5.81±0.05	7.48±0.19	52.67±0.82
	GG	5.76±0.03	7.27±0.32	51.40±1.37
	P-value	NS	NS	NS
	Mean	5.79B±0.03	7.34±0.17	52.26±0.74

• The means per column within the breed and genotype bearing different letters differ significantly at the 5% probability level.

Table 3. Effect of the strain and genotype of the CAPN1 gene on the physical characteristics of meat (pH, water holding capacity (WHC) and cooking loss).

Cooking loss

The cooking loss of CC and GG genotypes of site 316 for both Jenoubi and cross calves exceeded that of CG table 3. However, all calves' genotypes at site 530 showed similar cooking loss percentages.

Effect of calpain gene polymorphisms on sensory meat traits

Color

Table (4) indicated no significant effect due to different genotypes in sites 316 and 530 of Jenoubi calves. Cross calves GG genotype at 316 sites showed better ($P<0.05$) color value (8.39) than those of CC and CG (8.00 and 7.91, respectively).

Tenderness

Calpine gene polymorphisms had a significant ($P<0.05$) effect on tenderness of Jenoubi calves' meat Table 4. The highest tenderness value exhibited by the GG genotype (8.13) of the site was 316 in comparison with those of CC and CG (7.51 and 7.20, respectively). The results also showed significant ($P<0.05$) superiority of AG (7.60) of site 530 versus the GG genotype (7.34). A significant effect was also observed between the genotypes of the 316 sites of cross calves. GG and CC genotypes recorded higher ($P<0.05$) tenderness values (8.13 and 8.00 respectively) than that of CG (7.43). Besides, the AG genotype of site 530 of cross calves recorded a higher value (7.91) than that of the GG genotype (7.58). The overall mean of cross calves' meat tenderness exceeded that of Jenoubi.

Juiciness

It is noticeable from Table (4) that the genotypes CC and GG were significantly ($P<0.05$) superior to the genotype CG in the juiciness of both the Jenoubi and cross calves at site 316, where the values of CC, GG and CG were (7.83, 7.58, 7.20) and (8.13, 7.97 and 7.43) for both Jenoubi and cross calves respectively. The results in Table (4) also indicated that there is a significant ($P<0.05$) superiority of the AG genotype over the GG genotype for both the Jenoubi and cross calves at site 530. The values for the AG and (GG) genotypes were 7.66 and 7.39, respectively, for the Jenoubi and 7.89 and 7.58 for the cross. The averages of the cross claves outperformed those of the Jenoubi breed in juiciness.

General acceptance

Table (4) showed the significant superiority (P<0.05) of the polymorphisms of the calpain gene in the Jenoubi breed, as GG exceeds CC and CG at site 316 in grades of general acceptance. The results indicate a significant superiority (P<0.05) of GG and CC genotypes over the CG in the general acceptance of the cross claves at site 316. No significant differences existed between the genotypes within cross or Jenoubi calves at site 530. The overall mean of the cross calves exceeded that of Jenoubi breed calves in general acceptance.

Breed	Genotypes	Color	Tenderness	Juiciness	General Acceptance
	Site 316				
Jenoubi	GG	7.79±0.08	8.13a±0.32	7.58b±0.08	8.00a±0.26
	CG	7.80±0.30	7.20b±0.35	7.20b±0.31	7.40b±0.29
	CC	8.00±0.28	7.51b±0.10	7.83a±0.28	7.66b±0.08
Cross	GG	8.38a±0.11	8.13a±0.28	7.97a±0.12	7.90b±0.14
	CG	7.91b±0.15	7.43b±0.17	7.43b±0.15	7.90b±0.14
	CC	8.00b±0.24	8.00a±0.13	8.13a±0.25	8.25a±0.20
Jenoubi	Site 530				
	AG	7.86±0.09	7.60±0.08	7.66a±0.10	7.70±0.09
	GG	7.69±0.14	7.34±0.16	7.39b±0.14	7.60±0.13
Cross	AG	8.16±0.10	7.91a±0.11	7.89a±0.10	8.16±0.09
	GG	8.32±0.17	7.58b±0.16	7.58b±0.17	7.94±0.15
Jenoubi overall		7.86±0.14	7.51B±0.16	7.53B±0.16	7.69B±0.14
Cross overall mean		8.09±0.10	7.85A±0.11	7.84A±0.11	8.12A±0.09

• The means per column within the breed and genotype bearing different letters differ significantly at the 5% probability level.

Table 4. Effect of breed and genotypes of Calpain gene on meat sensory characteristics (color, tenderness, juiciness and general acceptance).

Discussion

The results indicated a significant effect between the means of the Jenoubi and the cross calves. The reason for the superiority of some genetic structures over others in water-holding capacity may be attributed to the increase in the activity of calpain, which breaks down structural proteins such as desmin, the contraction of muscle fibers decreases, and thus water loss from meat is reduced [15].

The good tenderness with an increase in absolute pH above 6.1 is mainly due to improvements in water holding capacity and consequent reduction in cooking losses [16].

The proteolytic calpain system plays a significant role in the proteolysis and tenderization of meat after slaughter, as confirmed by [17] that the leading role in the softening process is due to the enzyme encoded by the CAPN1 gene[19].

Conclusion

The results showed moderate to high allelic frequencies that correlate with the tenderness of the CAPN1 gene. There are relationships between genotypes of this

gene in two different sites (316 and 530). It might be used in the local Jenoubi breed selection program and their crosses.

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