Acta Scientiarum
http://periodicos.uem.br/ojs/acta
ISSN on-line: 1807-8672
Doi: 10.4025/actascianimsci.v47i1.71363

Associations between the genotypes of the calpain and calpastatin genes and performance, carcass parameters and meat quality in Nelore cattle

Claudiana Esteves Couto[1], Kalynka Gabriella do Livramento[2], Luciano Vilela Paiva[2], Ana Paula Peconick[1], Cesar Augusto Pospissil Garbossa[3], Melissa Siqueira Martins[1] and Peter Bitencourt Faria[1*]

[1]Departamento de Medicina Veterinária, Universidade Federal de Lavras, Av. Sul UFLA, Aquenta Sol, 37200-000, Lavras, Minas Gerais, Brasil. [2]Departamento de Química, Universidade Federal de Lavras, Lavras, Minas Gerais, Brasil. [3]Departamento de Nutrição Animal e Produção, Faculdade de Medicina Veterinária e Zootecnia, Universidade de São Paulo, Pirassununga, São Paulo, Brasil. *Author for correspondence. E-mail: peter@ufla.br

ABSTRACT. The objective of this study was to evaluate the associations between polymorphisms in the calpain (*CAPN*) and calpastatin (*CAST*) genes with the performance, carcass quality and meat quality traits of Nelore cattle. A total of 95 male Nelore cattle were used for the experiment. For molecular analysis, genomic DNA was extracted from the muscle tissue of the animals, and the polymerase chain reaction–single-strand conformation polymorphism (PCR–SSCP) technique was used to detect and identify the following polymorphisms: three genotypes of the *CAPN9* gene *(exon 9)*, eight patterns in the *CAPN14 gene (exon 14)* and four genotypes of the *CAST5* gene *(exon 5)*. The *CAST5* genotype was associated with soluble collagen content and the percentages of lauric (C12:0) and palmitic (C16:0) acids. The *CAPN14* genotype affected the final pH and moisture content of the Nellore cattle meat. *CAPN9* gene polymorphisms were not associated with the Nelore cattle parameters analyzed. The occurrence of *CAST5* and *CAPN14* gene polymorphisms may change the fatty acid profile and parameters associated with the quality of Nellore cattle meat.

Keywords: pH; collagen; fatty acids.

Received on February 20, 2024.
Accepted on November 14 2024.

Introduction

In Brazil, cattle herds are composed mostly of animals of Zebu origin, with Nelore animals being the main representative. Nelore is the main breed used for industrial cross-breeding for meat production due to its productive characteristics, adaptability and rusticity in a tropical climate through extensive, intensive or confined systems, accounting for more than 90% of Brazilian purebred or crossbred animal herds (Magalhães et al., 2016). It is essential to analyze the presence of polymorphisms in genes that are associated with performance parameters and carcass and meat quality for commercial breeds of cattle raised in Brazil, as it contributes to genetic improvement programs assisted by molecular markers to identify animals with the best traits for meat production. Such evaluations of different genes have shown the presence of genetic polymorphisms and identified mutations and genotypes in the different breeds and their crosses, which influence the phenotypic traits related to meat production by affecting the growth rate, muscle development and lipid metabolism (Magalhães et al., 2016; Kostusiak et al., 2023).

Among the genes evaluated, those associated with the calpain (*CAPN*) and calpastatin (*CAST*) complex stand out, as previous studies have revealed the associations between the presence of polymorphisms and tenderness indices in different cattle breeds (Parra-Bracamonte et al., 2015; Castro et al., 2016; Carvalho et al., 2017; López Rojas et al., 2017; Kök & Atalay, 2017; Lee et al., 2019; Zalewska et al., 2021; Pereira et al., 2022) and changes in parameters associated with beef quality (Casas et al., 2004; Schenkel et al., 2006; Curi et al., 2010; Upadhyay et al., 2021). Performance traits, such as intramuscular fat and carcass cover fat, also determine the organoleptic traits that influence meat quality (tenderness, flavor and acceptability) and are associated with the polymorphisms of these genes in different breeds, including Nellore (Pinto et al., 2010; Melucci et al., 2012; Saucedo-Uriarte et al., 2024).

The gene that encodes calpastatin in cattle is located on chromosome 7 and is composed of 35 exons, while the gene that encodes calpain in cattle is located on chromosome 29 (Smith et al., 2000; Corva et al., 2007;

Curi et al., 2009). In the present study, the *CAPN9* (exon 9) and *CAPN14* (exon 14) genes, which are related to the calpain/calpastatin system, were found to be associated with subcutaneous fat thickness in Wagyu × Limousin cattle according to Jiang et al. (2009); the water retention capacity of Nelore meat according to Tizioto et al. (2013); and muscle color and meat marbling in Hereford cattle according to Melucci et al. (2012). *CAST5* gene (exon 5) polymorphism is associated with variations in beef tenderness and is considered a genetic marker for this parameter (Curi et al., 2009).

Thus, the objective of this study was to verify the association of genotype based on polymorphisms of the calpastatin (*CAST5*) and calpain (*CAPN9* and *CAPN14*) genes with performance parameters, carcass traits and parameters associated with meat quality (physical parameters, chemical composition and lipid profile) in Nellore cattle.

Materials and methods

In this study, samples were collected from a bovine population of 95 male Nelore animals that were maintained in feedlots for 88 days and subsequently slaughtered. The samples were derived from animals receiving two isonutrient diets (with and without cottonseed) formulated to meet the daily weight gain requirements of the National Research Council (NRC, 2000), Table 1. This study was approved by the Committee for Ethics in the Use of Animals (CEUA) of the UFLA under registration number 040/12. The animals were weighed at the beginning and end of the confinement period to determine the initial and final weights, and the average daily gain (ADG) was also determined.

Table 1. Composition of cattle´s diet during the experimental period (Esteves et al., 2017).

Product/Ingredient	Diets (%Dry matter)	
	Control	Cotton Seed
Corn	45.76	52.91
Soybean meal	7.87	2.00
Premix	3.93	3.82
Whole cottonseed	-	15.90
Soybean peel	10.00	10.00
Silage Mombasa	-	15.37
Silage Sorghum	32.44	-
Calculated Composition		
DM (%)	65.88	75.59
OM (%DM)	92.36	92.23
TDN (%DM)	70.72	73.23
ME (Mcal kg^{-1})	2.60	2.70
CP (%DM)	11.32	11.60
MM (%DM)	3.71	3.95
Ca (%DM)	0.20	0.19
P (%DM)	0.24	0.30
EE (%DM)	3.03	5.72
NDF (%DM)	33.07	33.02
NFC (%DM)	46.54	44.53

*Assurance levels (per kg of product): Calcium (min) 118.00 g kg^{-1}; Calcium (max) 145.00 g kg^{-1}; Phosphorus (Min) 96.80 g kg^{-1}; Sulphur (Min) 38.00 g kg^{-1}; Cobalt (min) 66.00 mg kg^{-1}; Copper (min.) 1810.00 mg kg^{-1}; Iron (Min.). 2846.00 mg kg^{-1}; Iodine (min). 89,50 mg kg^{-1}; Manganese (min) 1774.50 mg kg^{-1}; Selenium (Min.) 14.90 mg kg^{-1}; Zinc (min) 4298.50 mg kg^{-1}; Fluorine (max) 968.00 mg kg^{-1}. DM: Dry matter; OM: Organic matter; TDN: Total digestible nutrients; ME: Metabolizable energy; CP: Crude protein; MM: mineral matter; Ca: Calcium; P: Phosphorus; EE: ethereal extract; NDF: neutral detergent fiber; NFC: non-fiber carbohydrates.

The animals were slaughtered after 12 hours of solid fasting under humane conditions (Brasil, 2021), and the carcasses were cooled for 24 hours at ± 1ºC and then weighed to determine the cold carcass weight (CCW) and cold carcass yield (CCY). The left side of each carcass was then cut at the 12^{th} rib to measure the rib eye area (REA) and subcutaneous fat thickness (SFT) in the *longissimus thoracis* muscle. After the measurements were taken, the muscle samples were collected, packed in polyethylene film, identified and frozen at -18ºC for transport and subsequent analyses of the molecular and physicochemical parameters, chemical composition and fatty acid profile.

For the genetic evaluation, genomic DNA was extracted from muscle samples collected according to the CTAB protocol for DNA extraction described by Stefanova et al. (2013). The concentration and purity of the extracted DNA were quantified by measuring the absorbance at 260 nm (A260) and 280 nm (A280) with a

NanoDrop ND-1000 ultraviolet/visible (UV/Vis) spectrophotometer. The samples were diluted with sterile water to obtain the final desired concentration of 10 ng DNA μL^{-1}. Then, the integrity gel was prepared, and the excellent quality of the genomic DNA was confirmed.

For DNA studies, *primers* were designed for the genes evaluated, as follows: *CAPN9* (exon 9) (500 bp) forward (5'GGACTCAGGCTTGAACAGAGG3') and reverse (5'GCATGAAGTCTCGGAAGGA3') (60°C); *CAPN14* (exon 14) (593 bp) forward (5'GTAGAAAGCCCTCCCCTGTC3') and reverse (5'TCAGAGCCTCACTCTCCTCA3') (61°C); and *CAST5* (exon 5) (393 bp) forward (5'GGATTATTATCAACCAGACACCAAC3') and reverse (5'CAATACCTGCTGATGCCACA3') (61ºC) using a software tool available online (http://www.bioinformatics.nl/cgi-bin/primer3plus/primer3plus.cgi) and according to the procedures described by Couto et al. (2022) for the polymerase chain reaction–single-strand conformation polymorphism (PCR–SSCP) technique.

Meat quality was evaluated by measuring the following parameters after cooling: final pH (24h), using a peagameter with a penetration probe (*Hanna Instruments*, HI 99163, Romania); color, using a colorimeter (Konica Minolta CM-700, Singapore) operating in the CIEL*a*b* system with an illuminator D65, observer angle of 10º and specular mode excluded (SCE), to obtain the lightness (L *), redness (a*) and yellowness (b*) indices, the saturation index (C*) and the hue angle (h*) according to the methodology of Ramos and Gomide (2017); coking loss (CL), by determining the difference in weight after cooking the samples on an electric plate until reaching a temperature of 72ºC American Meat Science Association (AMASA, 1978); and shear force (SF), which was determined from cooked samples that had been cut into pieces (1.0 × 1.0 cm) in the longitudinal direction of the muscle fibers and using a texturometer with a Warner–Bratzler probe (Chrystall & Devine, 1991). In addition, the myofibrillar fragmentation index (MFI) was evaluated on days 1 and 21; the soluble and insoluble fractions were determined; and total collagen was quantified according to the procedures described by Ramos and Gomide (2017). The chemical composition of the meat was evaluated by determining the moisture, protein, ether extract and ash contents Association of Official Analytical Chemists (AOAC, 1990).

To analyze the fatty acid profile, the lipids were extracted according to the methodology of Folch et al. (1957) and esterified according to Hartman and Lago (1973), as modified by Maia and Rodrigues-Amaya (1993). Fatty acid analysis was performed by gas chromatography on a Shimadzu CG 2010 gas chromatograph (*Agilent Technologies Inc., Palo Alto, CA, USA*) equipped with a flame ionization detector, a 1:50 split injector and a Supelco capillary column (SPTM-2560, 100 m × 0.25 mm × 0.20 µm; *Supelco Inc., Bellefonte, PA, USA*). The chromatographic temperature conditions were as follows: an initial temperature of 140ºC for 5 min., and increasing at a rate of 4ºC $min.^{-1}$ to 240ºC, where it was maintained for 30 min. for a total run time of 60 min. The injector and the detector were both maintained at a temperature of 260ºC. Helium was used as the carrier gas. Fatty acids were identified by comparing their retention times to those of the SupelcoTM37 chromatographic standard FAME Mix® (*Supelco Inc., Bellefonte, PA, USA*) and are expressed as a percentage (%) of the total fatty acids identified.

To verify the association between the polymorphism of each gene and the studied parameters, the genes and their respective genotypes were considered fixed effects. Diet (with and without the use of cottonseed) and animal age (groups of animals aged 13 to 24 months, 25 to 36 months and 37 to 48 months) were included as random factors. Each sample was considered an experimental unit for evaluating the associations between the genotypes of the genes and the parameters evaluated. The data were analyzed using the GLM procedure in SAS (2011), and genotypes were associated with the studied parameters according to the F test (α=0.05). The means were compared via Tukey's test (p=0.05). All evaluated variables were tested for normality using the Shapiro–Wilk test before analysis, and any variable that did not show a normal distribution was transformed using the SAS RANK procedure (2011).

Results

The PCR products of the genes *CAPN9, CAPN14 and CAST*5 were subjected to gel electrophoresis, and the DNA fragments were separated according to size, i.e., DNA fragments of the same length formed a single band with the same size in the gel for each specific gene. Therefore, these fragments were used directly for *PCR–SSCP* analysis. Among the samples analyzed from the animals in the present study, Couto et al. (2022) reported the presence of three genotypes (AC (n=53), BC (n=3) and AA (n=39)) and three alleles with frequencies (f(C) values) of A (0.6665), B (0.3050) and C (0.0285) for the *CAPN9* gene, while for the *CAPN14* gene, they reported eight genotypes (AG (n=15), EF (n=11), AC (n=14), BB (n=14), CE (n=14), DG (n=4), AH (n=5) and BI (n=18)) with nine different f(C) alleles: A (0.1880), B (0.1880), C (0.1600), D (0.0220), E (0.1435), F (0.0610), G (0.1050),

H (0.0275) and I (0.1050). According to these authors, two polymorphisms were identified for the *CAPN9* gene (c.5861G>A and c.5498A>G) and *CAPN14* (c.11054 T>C and c.11161C>G). For the *CAST5* gene, these authors reported the presence of four genotypes (BC (n=20), AC (n=53), BD (n=8) and EE (n=14)) with five f(C) alleles (0.3772), B (0.1363), C (0.3772), D (0.0410) and E (0.0682)) and four polymorphisms (c.29919C>T; c.29963A>C; c.29978C>A; and c.30019G>T). Thus, based on these previous results, we sought to identify which genotype of each gene could influence the parameters studied.

Statistical analysis revealed no associations between the genotypes identified for each gene and the performance and carcass parameters of the Nellore cattle (Table 2). Regarding the physicochemical properties and chemical composition of the meat, the *CAST5* gene influenced the soluble collagen content (p=0.019), while the *CAPN14* gene influenced the final pH (p=0.022) and collagen and moisture contents (p=0.004), Table 3. For the *CAPN9* gene, no associations were observed between any of the genotypes found and the parameters evaluated.

Table 2. Statistical analysis of the performance parameters and carcass composition of Nelore cattle as a function of the *CAPN9, CAPN14* and *CAST5* genes.

Parameters	*CAPN9*			*CAPN14*			*CAST5*		
	Mean	SEM	P value*	Mean	SEM	P value*	Mean	SEM	P value*
Initial weight (kg)	396.8	32.6	0.372	385.2	28.6	0.813	386.2	20.2	0.937
Final weight (kg)	539.1	42.0	0.784	528.2	36.2	0.370	530.3	25.4	0.390
ADG (kg day^{-1})	1.6	0.1	0.672	1.6	0.2	0.260	1.6	0.1	0.367
CCW (kg)	287.9	24.2	0.807	283.1	20.8	0.520	284.8	14.6	0.688
CCY (%)	53.3	1.0	0.943	53.5	0.9	0.854	53.7	0.6	0.473
REA (cm^2)	73.0	6.6	0.652	70.1	5.7	0.217	71.6	4.0	0.288
SFT (mm)	6.3	1.6	0.839	6.6	1.3	0.297	6.6	1.0	0.938

*F test (α=0.05); SEM - standard error of the mean; CCW - cold carcass weight; CCY - cold carcass yield; REA - rib eye area; SFT- subcutaneous fat thickness.

Table 3. Statistical analysis of the quality parameters (physicochemical and centesimal composition) of meat from Nelore cattle as a function of the *CAPN9, CAPN14* and *CAST5* genes.

Parameters	*CAPN9*			*CAPN14*			*CAST5*		
	Mean	SEM	P value*	Mean	SEM	P value*	Mean	SEM	P value*
Physicochemical									
pH	5.7	0.16	0.306	5.6	0.13	0.022	5.7	0.102	0.362
L*	27.1	2.18	0.238	27.0	1.81	0.088	26.4	1.391	0.889
a*	18.1	2.42	0.479	18.3	2.10	0.902	17.9	1.367	0.225
b*	16.9	1.59	0.123	17.0	1.39	0.671	16.5	0.994	0.135
C*	24.8	2.78	0.319	25.0	2.42	0.860	24.4	1.720	0.177
h*	43.1	1.89	0.651	42.9	1.63	0.702	42.9	1.193	0.909
CL (%)	29.8	8.60	0.077	31.9	7.41	0.559	32.0	5.321	0.424
SF (kgf)	11.4	1.97	0.542	11.4	1.71	0.763	11.2	1.242	0.667
MFI T1	496.1	29.88	0.444	490.5	25.25	0.379	499.1	18.746	0.750
MFI T21	427.5	30.45	0.750	429.8	25.78	0.482	437.3	18.596	0.146
SCC	0.3	0.09	0.636	0.3	0.08	0.507	0.3	0.056	0.019
ICC	1.8	0.69	0.560	1.7	0.59	0.916	1.8	0.432	0.830
TCC	2.1	0.72	0.885	2.0	0.63	0.989	2.1	0.453	0.681
Centesimal composition (%)									
Ash	1.3	0.15	0.591	1.3	0.13	0.619	1.3	0.095	0.248
Moisture	75.2	0.55	0.859	75.2	0.43	0.004	75.3	0.339	0.253
Ethereal Extract	3.2	0.63	0.849	3.2	0.54	0.778	3.1	0.390	0.270
Protein	18.0	0.96	0.770	18.1	0.79	0.178	17.9	0.601	0.715

F test (α=0.05); SEM - standard error of the mean; SF - shear force; CL - cooking loss; L - lightness index; a*, redness index; b*, yellowness index; c*, saturation index - chroma; h* - hue angle; MFI T1, myofibrillar fragmentation index at time 1 day; MFI T21 - myofibrillar fragmentation index at 21 days; TCC - total collagen content (mg g^{-1}); ICC - insoluble collagen content (mg g^{-1}); SCC - soluble collagen content (mg g^{-1}).

The evaluation of the lipid profile revealed that there was an association between the genotypes of the *CAST5* gene with the fatty acids lauric acid (C12:0) (p=0.006) and palmitic acid (C16:0) (p=0.032) (Table 4). However, no associations were found between the presence of polymorphisms of the other genes and the fatty acid profile.

For the *CAST5* gene, the animals that presented the highest soluble collagen contents were identified to have the BC genotype, while the lowest soluble collagen contents were found with the BD, and AC and EE genotypes had intermediate results. For this gene, the EE genotype presented the highest contents of C12:0 and C16:0 fatty acids, while animals with the BC and AC genotypes had lower mean levels of C16:0, and lower values of C12:0 were observed with the BC and BD genotypes (see Table 5).

Table 4. Statistical analysis of the influence of genotype the of *CAPN9*, *CAPN14 and CAST5* genes on the fatty acid profile of meat from Nelore cattle.

Fatty acids (%)	*CAPN9*			*CAPN14*			*CAST5*		
	Mean	SEM	P value*	Mean	EPM	P value*	Mean	EPM	P value*
C10:0	0.10	0.04	0.182	0.09	0.03	0.285	0.10	0.02	0.250
C12:0	0.10	0.02	0.546	0.10	0.02	0.835	0.11	0.01	0.006
C14:0	2.40	0.28	0.848	2.43	0.24	0.815	2.51	0.17	0.147
C14:1	0.41	0.08	0.753	0.40	0.07	0.518	0.43	0.05	0.314
C15:0	0.33	0.05	0.875	0.33	0.04	0.675	0.34	0.03	0.149
C16:0	24.49	0.54	0.667	24.46	0.45	0.264	24.66	0.32	0.032
C16:1	3.00	0.20	0.516	2.96	0.17	0.522	3.00	0.13	0.841
C17:0	0.91	0.09	0.821	0.90	0.08	0.895	0.92	0.06	0.346
C17:1	0.68	0.08	0.837	0.68	0.07	0.911	0.68	0.05	0.358
C18:0	14.93	1.69	0.833	15.06	1.46	0.961	15.26	1.06	0.897
C18:1ω9t	1.73	0.34	0.649	1.66	0.29	0.933	1.71	0.21	0.412
C18:1ω9c	39.92	1.75	0.649	39.46	1.47	0.472	39.44	1.10	0.731
C18:2ω6t	0.11	0.02	0.983	0.11	0.02	0.980	0.11	0.01	0.911
C18:2ω6c	8.72	2.59	0.911	9.15	2.22	0.948	8.55	1.62	0.615
C20:0	0.15	0.01	0.889	0.15	0.01	0.773	0.15	0.01	0.372
C18:3ω6	0.03	0.01	0.383	0.03	0.01	0.343	0.03	0.01	0.085
C20:1	0.31	0.07	0.784	0.31	0.06	0.335	0.30	0.04	0.767
C18:3ω3	0.50	0.08	0.767	0.51	0.07	0.959	0.51	0.05	0.626
C20:2	0.18	0.06	0.918	0.17	0.05	0.349	0.18	0.04	0.707
C20:3ω6	0.15	0.04	0.937	0.15	0.03	0.953	0.15	0.02	0.881
C22:1ω9	0.01	0.06	0.473	0.02	0.05	0.208	0.01	0.86	0.508
C20:4ω6	0.56	0.17	0.356	0.58	0.15	0.370	0.58	0.11	0.524
C20:5ω3	0.13	0.05	0.632	0.13	0.05	0.835	0.13	0.03	0.235
SFA	43.53	2.29	0.791	43.66	1.99	0.938	44.17	1.42	0.453
MUFA	46.07	1.94	0.421	45.49	1.65	0.615	45.57	1.22	0.846
PUFA	10.38	2.72	0.878	10.83	2.35	0.971	10.24	1.70	0.684
∑ω3	0.63	0.11	0.942	0.64	0.09	0.951	0.64	0.07	0.856
∑ω6	9.58	2.66	0.899	10.02	2.29	0.968	9.42	1.66	0.644

*F test (α=0.05); SEM - standard error of the mean; SFA - sum of total saturated fatty acids (%); MUFA - sum of total monounsaturated fatty acids (%); PUFA - sum of total polyunsaturated fatty acids (%); ∑ω3, sum of total omega 3 series fatty acids (%); ∑ω6, sum of total omega 6 series fatty acids (%).

Table 5. Physicochemical parameters, centesimal composition and lipid profile as a function of *CAST5* and *CAPN14* genotypes.

Variable	*CAST5* genotype *			
	BC	DB	CA	EE
SCC	0.406a	0.213b	0.308ab	0.303ab
C12:0	0.09b	0.09b	0.10ab	0.14a
C16:0	24.38b	24.50ab	24.49b	25.26a

Variable	*CAPN14* genotype *							
	AC	AG	AH	BB	BI	CE	DG	EF
pH	5.54b	5.58ab	5.45b	5.75ab	5.70ab	5.56ab	5.55ab	5.87a
Moisture	74.38b	74.93ab	75.30ab	75.65a	75.57a	75.01ab	75.40ab	75.44ab

*Tukey's test (α=0.05); *CAPN14* - effect of genotype on the *CAPN14* gene; *CAST5* - effect of genotype on the *CAST5 gene;* SCC - soluble collagen content (mg g^{-1}).

For the calpain gene (*CAPN14*), the animals that presented the highest mean final pH were identified as having the EF genotype, while the AC and AH genotypes had lowest pH values. The highest moisture content in the meat was found with the BB and BI genotypes, and the lowest moisture content was found with the AC genotype (Table 5).

Discussion

The results of the present study indicate the presence of polymorphisms and the formation of genotypes for the genes studied in Nelore cattle, which confirms the results found in the literature for cattle of different genetic groups and breeds (Xin et al., 2011; Avilés et al., 2013; Li et al., 2013; Blecha et al., 2019).

Considering the results for the *CAPN9* and *CAPN14* genes for the animals in the present study, it would be possible to find more genotypes and alleles (Couto et al., 2022). This finding differs from the results previously reported for the *CAPN14* gene by Trujano-Chavez et al. (2021) for Braunvieh animals with two genotypes and two alleles, one of which had a frequency of 0.93, and the report of Carvalho et al. (2017), who also found only two genotypes and two alleles with frequencies of 0.9917 and 0.9261 for the *CAPN9* and *CAPN14* genes in the

Nelore cattle population. For the *CAPN9* gene in Aceh cattle, Rosa et al. (2020) reported a similar result to that in the present study, with the presence of polymorphisms and the formation of three genotypes from two alleles with a higher prevalence of 0.86. Thus, it appears that for these genes, unlike previous data in the literature that indicated a higher rate of homozygosity, in the population of Nelore cattle studied, there was greater genetic variation, with eight genotypes for *CAPN14*, three for *CAPN9*, and one for which the number of alleles did not demonstrate an individual frequency above 0.70.

Despite the low genetic variation in calpain genes being observed in other cattle populations, the influence of the identified genotypes on certain meat quality parameters in the *longissimus dorsi* muscle has been reported, such as the color indices L*, a* and b* and marbling in Hereford cattle for the *CAPN9* gene (Melucci et al., 2012); marbling and intramuscular fat content of Bali cattle for the *CAPN1* gene (Dairoh et al., 2020); and the association between *CAPN14* gene polymorphism and meat color in terms of the a* (Castro et al., 2016) and b* values (Ribeca et al., 2012).

In the present study, an association was observed between *CAPN14* genotype and the final moisture and pH value of meat from the studied population of Nelore cattle. Xin et al. (2011), evaluating *CAPN* gene polymorphisms (exon 17) in Chinese cattle, reported similar results, with an association of the AA genotype with lower pH values (5.45) at the beginning of maturation in the *post mortem* period (0, 1, and 3 days) and genotype BB, which had a higher pH value (5.64) after 7 days of storage. The rate of decrease in muscle pH is one of the most important factors that affects meat tenderness, as it influences *post mortem* proteolysis. Meat with an intermediate pH (5.8 < pH <6.2) has a lower rate of tenderization, probably because of the reduced degradation of myofibrillar proteins, which is associated with a reduction in calpastatin activity and greater calpain activation (Hwang & Thompson, 2001; Contreras-Castillo et al., 2016). Although there was no change in the meat tenderness or fragmentation index in the present study, the difference in pH values between animals was greater than 5%, which could influence other quality parameters, especially color, which is the main attribute of consumer choice (Weglarz, 2010). This was verified by Ribeca et al. (2012) in Piemontese cattle in terms of water holding capacity and color.

Likewise, lower pH values may cause more water flow out of the muscle due to the reduction in isoelectric point, promoting the reduction in the space between actin and myosin and promoting greater exudation. Here, the animals with the AC genotype had lower pH values and a lower moisture content in the meat. Thus, variations in this parameter could also cause changes in meat juiciness due to reduced water retention in addition to the greater water loss that occurs during the preparation process (Warner, 2017).

However, some authors have found an association between genotypes derived from polymorphisms of calpain genes and the meat tenderness indices of Nelore (Pinto et al., 2010) and Creole (Saucedo-Uriarte et al., 2024) cattle. However, in the present study, this association was not observed, which is similar to what was reported by Leal-Gutiérrez et al. (2018), despite the presence of polymorphisms in both genes in cattle from the crossing of Angus and Brahman.

Regarding the performance and carcass parameters, no associations were detected between the identified genotypes of the calpain genes *CAPN9* and *CAPN14* and these parameters, which was also observed by Dairoh et al. (2021) in relation to ADG, ribeye area and subcutaneous fat thickness of Bali cattle.

In the studied cattle population, polymorphisms were observed, as was the formation of distinct genotypes for the calpastatin gene (*CAST5*), which has been reported for other cattle breeds and sheep by Kaplan and Atalay (2017). Similar to findings reported by other authors, in the present study, associations between *CAST5* gene genotype and tenderness index (Curi et al., 2009b; Leal-Gutiérrez et al., 2018), parameters of the carcass (ribeye area and subcutaneous fat thickness), chemical composition (intramuscular fat content) or meat tenderness for Nellore cattle and their crosses (Curi et al., 2009b) were not observed.

An association between *CAST5* gene genotype and lauric (C12:0) and palmitic (C16:0) acids was observed. Similar observation was also noted by Dunner et al. (2013), who reported an association between the *CAST* gene and the total C16:0 and C18:0 fatty acids in cattle of European origin from 15 different crosses. Li et al. (2013), who evaluated the association between single-nucleotide polymorphism (SNPs) of the *CAST* gene (intron 3) with meat quality traits of Yanbian cattle, found an effect on the fatty acid content, in which the AA and BB genotypes had higher levels of palmitic (C16:0) and linoleic acids (C18:2ω6). Aali et al. (2017), who assessed the relationship between the calpastatin gene (*CAST* - intron 5) and the fatty acid profile in Iranian sheep breeds, also identified an association between the genotype of this gene and the palmitic acid content (C16:0) in the *longissimus dorsi* muscle. According to these authors, the association of the calpastatin gene

with the composition of fatty acids is due to a possible pleiotropic effect of this gene that occurs due to the linkage disequilibrium of the quantitative trait loci (QTLs) within the gene itself with the QTLs close to the genes that biochemically influence the fatty acid profile of meat; thus, this gene had multiple effects on lipid composition.

Considering nutritional aspects, the animals with the EE genotype gave meat with higher amounts of fatty acids with a hypercholesterolemic effect. Consuming foods with more palmitic and lauric acids promotes an increase in blood cholesterol or in its total concentration in the plasma in the form of low-density lipoprotein (LDL), which may affect human health through hypercholesterolemia (DiNicolantonio & O'Keefe, 2018; Banskalieva et al., 2020).

The *CAST5* gene genotype was associated with the amount of soluble collagen. Collagen solubility is related to the presence of intermolecular interactions, called crosslinks, which promote the thermal stability of collagen and are generally associated with changes in meat tenderness (Pflanzer & Felicio, 2009; Lawrence et al., 2011; Silva et al., 2014).

There was a 47% difference in the amount of soluble collagen among the genotypes (Table 4). Nevertheless, there was no significant difference in shear force ($p=0.667$), even though there was a difference of 9.0% in relation to the values determined for the identified genotypes. Avilés et al. (2013) verified the association between *CAST* gene genotype and raw meat texture in Limousin animals, while in terms of shear force, which is analyzed from cooked meat, they did not find an association between the genotype of this gene and this parameter, even with a difference of 15%. According to these authors, other factors related to muscle composition might influence the shear force after cooking, reducing the observed effect of the genotypes.

In general, it is possible to verify the presence of polymorphisms of the *CAST5* and *CAPN14* genes and their associations with the physicochemical parameters, centesimal composition and lipid profile of Nelore cattle meat, as reported with other groups of animals of different races and genetic groups. However, considering the number of animals used in the present study, further complementary studies should be performed to explore the presence of polymorphisms in these genes and their relationships with phenotypic factors in a larger population of Nelore cattle to validate these findings.

Conclusion

The presence of *CAST5* gene polymorphisms was associated with changes in the composition of fatty acids and the amount of soluble collagen, while for the *CAPN14* gene, there were differences between the final pH and moisture content. *CAPN9* gene polymorphism was not associated with parameters related to meat production in Nellore cattle.

Acknowledgments

The authors thank the Foundation for Research Support of the State of Minas Gerais (FAPEMIG) for financial support of the project and the Coordination for the Improvement of Higher Education Personnel (CAPES) for providing the author with a scholarship.

References

Aali, M., Moradi-Shahrbabak, H., Moradi-Shahrbabak, M., Sadeghi, M., & Yousefi, A. R. (2017). Association of the calpastatin genotypes, haplotypes, and SNPs with meat quality and fatty acid composition in twoo Iranian fat-and thin-tailed sheep breeds. *Small Ruminant Research, 149*, 40-51. https://doi.org/10.1016/j.smallrumres.2016.12.026

American Meat Science Association [AMASA]. (1978). *Guidelines for cooking sensory evaluation of meat*. National Live Stock and Meat Board.

Association of Official Analytical Chemists [AOAC]. (1990). *Official methods of analysis of the Association of Official Analytical Chemists* (15th ed.) Arlington.

Avilés, C., Juárez, M., Peña, F., Domenech, V., Clemente, I., & Molina, A. (2013). Association of single nucleotide polymorphisms in CAPN1 and CAST genes with beef tenderness from Spanish commercial feedlots. *Czech Journal of Animal Science, 58*(10), 479-487. https://doi.org/10.17221/6997-CJAS

Banskalieva, V., Sahlu, T., & Goetsh, A.L. (2000). Fatty acid composition of goat muscles and fat depots: a review. *Small Ruminant Research, 37*(3), 255-268. https://doi.org/10.1016/S0921-4488(00)00128-0

Blecha, I. M. Z., Sousa, I. I., Ferreira, A. B. R., Feijó, G. L. D., Torres Júnior, R. A. A., & Siqueira, F. (2019). Alternative methodologies for genotyping polymorphisms in the *CAST* and *CAPN1* genes in beef cattle. *Revista Brasileira de Zootecnia, 48*, e20180218. https://doi.org/10.1590/rbz4820180218

Brasil. (2021). Ministério da Agricultura, Pecuária e Abastecimento. Portaria nº 365, de 16 de julho de 2021. Regulamento Técnico de Manejo Pré-abate e Abate Humanitário e os métodos de insensibilização autorizados pelo Ministério da Agricultura, Pecuária e Abastecimento. Diário Oficial da República Federativa do Brasil, Brasília, 2021. https://www.in.gov.br/en/web/dou/-/portaria-n-365-de-16-de-julho-de-2021-334038845

Carvalho, M. E., Eler, J. P., Bonin, M. N., Rezende, F. M., Biase, F. H., Meirelles, F. V., Regitano, L. C. A., Coutinho, L. L., Balieiro, J. C. C., &Ferraz, J. B. S. (2017). Genotypic and allelic frequencies of gene polymorphisms associated with meat tenderness in Nellore beef cattle. *Genetic Molecular Research, 16*(1), gmr16018957.

Casas, E., White, S. N., Riley, D. G., Smith, T. P. L., Brenneman, R. A., Olson, T. A., Johnson, D. D., Coleman, S. W., Bennett, G. L., & Chase Jr, C. C. (2005). Assessment of single nucleotide polymorphisms in genes residing on chromosomes 14 and 29 for association with carcass composition traits in Bos indicus cattle. *Journal of Animal Science, 83*(1), 13-19. https://doi.org/10.2527/2005.83113x

Chrystall, B. B., & Devine, C. E. (1991). *Quality assurance for tenderness* (Meat Industries Research Institute of New Zealand, Report n. 872, p. 23). Mirinz Publication.

Contreras-Castillo, C. J., Lomiwes, D., Wu, G., Frost, D., & Farouk, M. M. (2016). The effect of electrical stimulation on post mortem myofibrilar protein degradation and small heat shock protein kinetics in bull beef. *Meat Science, 113*, 65-72. https://doi.org/10.1016/j.meatsci.2015.11.012

Corva, P., Soria, L., Schor, A., Villarreal, E., Cenci, M. P., Motter, M., Mezzadra, C., Melucci, L., Miquel, C., Pavá n, E., Depetris, G., Santini, F., &Naón, J. G. (2007). Association of CAPN1 and CAST gene polymorphisms with meat tenderness in Bos taurus beef cattle from Argentina. *Genetics and Molecular Biology, 30*(4), 1064-1069. https://doi.org/10.1590/S1415-47572007000600006

Couto, C. E., do Livramento, K. G., Paiva, L. V., Peconick, A. P., Garbossa, C. A. P., Cardoso, T. B., Vasconcellos, R. C. C., & Faria, P. B. (2022). Identification of SNPs and changes in protein coding of genes associated with beef quality in Nellore cattle. *Genetics and Molecular Research, 21*(2), gmr18983.

Curi, R. A., Chardulo, L. A. L., Mason, M. C., Arrigoni, M. D. B., Silveira, A. C., & De Oliveira, H. N. (2009). Effect of single nucleotide polymorphisms of CAPN1 and CAST genes on meat traits in Nellore beef cattle (Bos indicus) and in their crosses with Bos taurus. *Animal Genetics, 40*(4), 456-462. https://doi.org/10.1111/j.1365-2052.2009.01859.x

Curi, R. A., Chardulo, L. A. L, Giusti, J., Silveira, A. C., Martins, C. L., & Oliveira, H. N. (2010). Assessment of GH1, CAPN1 and CAST polymorphisms as markers of carcass and meat traits in Bos indicus and Bos taurus–Bos indicus cross beef cattle. *Meat Science, 86*(4), 915-920. https://doi.org/10.1016/j.meatsci.2010.07.016

Curi, R. A., Salinas Fortes, M. R., Chardulo, L. A. L., Silveira, A. C., Arrigoni, M. de B., Martins, C. L., D' Avila Assumpção, M. E. O., & de Oliveira, H. N. (2009). Genetic polymorphisms related to meat traits in purebred and crossbred Nelore cattle. *Pesquisa Agropecuária Brasileira, 44*(12), 1660-1666. https://doi.org/10.1590/S0100-204X2009001200015

Dairoh, J., Ulum, M. F., Ishak, A. B. L, & Sumantri, C. (2020). Association of SNP g.232 G>T calpain gene with growth and live meat quality prediction using ultrasound images in bali cattle. *JITV, 26*(2), 49-56. https://doi.org/10.14334/jitv.v26i2.2701

DiNicolantonio, J. J., & O'Keefe, J. H. (2018). Effects of dietary fats on blood lipids: a review of direct comparison trials. *Open Heart, 5*(2), e000871. https://doi.org/10.1136/openhrt-2018-000871

Dunner, S., Sevane, N., Garcia, D., Cortés, O., Valentini, A., Williams, J. L., Mangin, B., Cañón, J., & Levéziel, H. (2013). Association of genes involved in carcass and meat quality traits in 15 European bovine breeds. *Livestock Science, 154*(1-3), 34-44. https://doi.org/10.1016/j.livsci.2013.02.020

Folch, J., Lees, M., & Sloane Stanley, G.H. (1957). A simple method for isolation and purification of total lipids from animal tissues. *Journal of Biological Chemistry, 226*(91), 497-509. https://doi.org/10.1016/S0021-9258(18)64849-5

Esteves, C., Varaschin, M. S., Garbossa, C. A. P., Marçal, J. O., Carvalho, F. P. de, & Faria, P. B. (2017). Performance, carcass parameters, meat quality and lipid profile from feedlot young bulls fed cottonseed.

Revista Brasileira de Saúde e Produção Animal, *18*(2), 347-358. https://doi.org/10.1590/S1519-99402017000200013

Hartman, L., & Lago, R. C. (1973). Rapid preparation to fatty acids methyl esters. *Laboratory Practice, 22*(6), 475-476. https://www.researchgate.net/publication/18441624

Hwang, I. H., & Thompson, J. M. (2001). The interaction between pH and temperature decline early postmortem on the calpain system and objective tenderness in electrically stimulated beef longissimus dorsi muscle. *Meat Science, 58*(2), 167-174. https://doi.org/10.1016/S0309-1740(00)00147-9

Jiang, Z., Michal, J.J., Chen, J., Daniels, T.F., Kunej, T., Garcia, M.D., ... MacNeil, M.D. (2009). Discovery of novel genetic networks associated with 19 economically important traits in beef cattle. *International Journal of Biological Science, 5*(6), 528-542. https://doi.org/10.7150/ijbs.5.528

Kaplan, S., & Atalay, S. (2017). Determination of Calpastatin Gene Polymorphism in Kıvırcık Crossbred Ewes by PCR-RFLP Method. *Selcuk Journal of Agriculture and Food Sciences, 31*(3), 147-150. https://doi.org/10.15316/SJAFS.2017.47

Kök, S., & Atalay, S. (2018). The use of various SNPs in CAST and CAPN1 genes to determine the meat tenderness in Turkish Grey Cattle. *Kafkas Üniversitesi Veteriner Fakültesi Dergisi, 24*(1), 1-8. https://doi.org/10.9775/kvfd.2017.17617

Kostusiak, P., Słósarz, J., Goł¸ebiewski, M., Grodkowski, G., & Puppel, K. (2023). Polymorphism of Genes and Their Impact on Beef Quality. *Current Issues in Molecular Biology, 45*(6), 4749-4762. https://doi.org/10.3390/cimb45060302

Lawrence, T. E., Whatley, J. D., Montgomery, T. H., Perino, L. J., & Dikeman, M. E. (2011). Influence of dental carcass maturity classification on carcass traits and tenderness of longissimus steaks from commercially fed cattle. *Journal of Animal Science, 79*(8), 2092-2096. https://doi.org/10.2527/2001.7982092x

Lee, H. J., Jin, S., Kim, H. J., Bhuiyan, M. S. A., Lee, D. H., Lee, S. H., Jang, S. B., Han, M. H., Lee, S. H., &Lee, S. H. (2019). Validation Study of SNPs in CAPN1-CAST Genes on the Tenderness of Muscles (Longissimus thoracis and Semimembranosus) in Hanwoo (Korean Cattle). *Animals, 9*(9), 691. https://doi.org/10.3390/ani9090691

Li, Y. X., Jin, H. G., Yan, C. G., Seo, K. S., Zhang, L. C., Ren, C. Y., & Jin, X. (2013). Association of CAST gene polymorphisms with carcass and meat quality traits in Yanbian cattle of China. *Molecular Biology Reports, 40*, 1875-1881. https://doi.org/10.1007/s11033-012-2243-2

López Rojas, L. E., Patiño-Cadavid, L., López Herrera, A., & Echeverri Zuluaga, J. J. (2017). Variabilidad genética en seis SNPs de los genes CAPN1, CAST y LEP de toros brahman en ganaderías del trópico bajo colombiano. *CES Medicina Veterinaria y Zootecnia, 12*(2), 88-102. https://doi.org/10.21615/cesmvz.12.2.2

Magalhães, A. F. B., Camargo, G. M. F, Fernandes, G. A., Junior Gordo, D. G. M., Tonussi, R. L., Costa, R. B, Espigolan, R., Silva, R. M. O., Bresolin, T., Andrade, W. B. F., Takada, L., Feitosa, F. L. B., Baldi, F. Carvalheiro, F. R., Chardulo, L. A. L., & Albuquerque, L. G. (2016) Genome-wide association study of meat quality traits in nellore cattle. *PLoS One, 11*(6), e0157845. https://doi.org/10.1371/journal.pone.0157845

Maia, E. L., & Rodrigues-Amaya, D. B. (1993) Avaliação de um método simples e econômico para a metilação de ácidos graxos com lipídios de diversas espécies de peixes. *Revista do Instituto Adolfo Lutz*, *53*(1-2), 27-35. https://doi.org/10.53393/rial.1993.53.35982

Melucci, L. M., Panarace, M., Feula, P., Villarreal, E. L., Grigioni, G., Carduza, F., Soria, L. A., Mezzadra, C. A., Arceo, M. E., Mazzucco, J. P., Corva, P. M., Irurueta, M., Rogberg-Muñoz, A., & Miquel, M. C. (2012). Genetic and management factors affecting beef quality in grazing Hereford steers. *Meat Science, 92(4),* 768-774. https://doi.org/10.1016/j.meatsci.2012.06.036

National Research Council [NRC]. (2000). *Nutrient requirements of beef cattle* (7th ed. revised). The National Academies Press. https://doi.org/10.17226/9791

Parra-Bracamonte, G. M., Martínez-González, J. C., Sifuentes-Rincón, A. M., Moreno-Medina, V. R., & Ortega-Rivas, E. (2015). Meat tenderness genetic polymorphisms occurrence and distribution in five Zebu breeds in Mexico. *Electronic Journal of Biotechnology, 18*(5), 365-367. https://doi.org/10.1016/j.ejbt.2015.07.002

Pereira, J. A. C., Falomir-Lockhart, A. H., Loza, A. J., Villegas-Castagnasso, E. E., Rojas, P., Carino, M. H., Rodrigo Hoyos, R., Rogberg-Muñoz, A., & Giovambattista, G. (2022). Genetic diversity of Calpain 1 gene in Creole, Nellore and Brahman bovine breeds in Bolivia. *Archivos Latinoamericanos de Produccion Animal, 30*(2), 121-132. https://doi.org/10.53588/alpa.300206

Pflanzer, S. B., & Felicio, P. E. D. (2009). Effects of teeth maturity and fatness of Nellore (Bos indicus) steer carcasses on instrumental and sensory tenderness. *Meat Science, 83*(4), 697-701. https://doi.org/10.1016/j.meatsci.2009.08.011

Pinto, L. F., Ferraz, J. B., Meirelles, F. V., Eler, J. P., Rezende, F. M., Carvalho, M. E., Almeida, H. B., & Silva, R. C. (2010). Association of SNPs on CAPN1 and CAST genes with tenderness in Nellore cattle. *Genetic Molecular Research, 9*(3), 1431-1442.

Ramos, E. M., & Gomide, L. A. M. (2017). *Avaliação da qualidade de carnes - Fundamentos e Metodologias* (2a ed.). Editora UFV.

Ribeca, C., Bonfatti, V., Cecchinato, A., Albera, A., Maretto, F., Gallo, L., & Carnier, P. (2012). Association of polymorphisms in calpain 1, (mu/I) large subunit, calpastatin, and cathepsin D genes with meat quality traits in double-muscled Piemontese cattle. *Animal Genetics, 44*(2), 193-196. https://doi.org/10.1111/j.1365-2052.2012.02370.x

Rosa, T. S., Azhar, A., Akmal, M., Hambal, M., Lubis, T. M., Ferasyi, T. R., & Santosa, S. F. (2020). Single Nucleotide Polymorphism (SNP) 316 on Calpain Gene in Aceh Cattle. *E3S Web of Conferences*, *151*, 01027. https://doi.org/10.1051/e3sconf/202015101027

SAS Institute. (2011). *Statistical Analysis System*: *user guide - version 9.2* [CD-ROM]. SAS Insitute Inc.

Saucedo-Uriarte, J. A., Portocarrero-Villegas, S., Diaz-Quevedo, C., Quispe-Ccasa, H. A., Tapia-Limonchi, R., Chenet, S. M., Cesar, A. S. M., & Cayo-Colca, I. S. (2024). Association of polymorphisms in CAPN1 and CAST genes with the meat tenderness of Creole cattle. *Scientia Agricola, 81*, e20230098. https://doi.org/10.1590/1678-992X-2023-0098

Schenkel, F. S., Miller, S. P., Jiang, Z., Mandell, I. B., Ye, X., Li, H., & Wilton, J. W. (2006) Association of a single nucleotide polymorphism in the calpastatin gene with carcass and meat quality traits of beef cattle. *Journal of Animal Science, 84*(2), 291299. https://doi.org/10.2527/2006.842291x

Silva, B., Poleti, M. D., Moncau, C. T., Rosa, A. F., Silva, S. L. E., & Balieiro, J. C. C. (2014). Neuroendocrine, metabolic and meat quality traits in castrated and non-castrated Nellore cattle. *Ciência Rural, 44*(5), 904-910. https://doi.org/10.1590/S0103-84782014005000003

Stefanova, P., Taseva, M., Georgieva, T., Gotcheva, V., & Angelov, A. A. (2013). Modified CTAB Method for DNA Extraction from Soybean and Meat Products. *Biotechnology & Biotechnological Equipment, 27*(3), 3803-3810. https://doi.org/10.5504/BBeQ.2013.0026

Tizioto, P. C., Decker, J. E., Taylor, J. F., Schnabel, R. D., Mudadu, M. A., Silva, F. L., Mourão, G. B., Coutinho, L. L., Tholon, P., Sonstegard, T. S., Rosa, A. N., Alencar, M. M., Tullio, R. R., Medeiros, S. R., Nassu, R. T., Feijó, G. L. D., Silva, L. O. C., Torres, R. A., Siqueira, F., R. H. Higa, & Regitano, L. C. A. (2013). Genome scan for meat quality traits in Nelore beef cattle. *Physiological Genomics, 45*(21), 1012-1020. https://doi.org/10.1152/physiolgenomics.00066.2013

Trujano-Chavez, M. Z., Valerio-Hernández, J. E., López-Ordaz, R., Pérez-Rodríguez, P., & Ruíz-Flores, A. (2021). Allelic and genotypic frequencies for loci associated with meat quality in Mexican Braunvieh cattle. *Tropical Animal Health and Production,* 53(307). https://doi.org/10.1007/s11250-021-02757-5

Upadhyay, M., Derks, M. F. L., Andersson, G., Medugorac, I., Groenen, M. A. M., & Crooijmans, R. P. M. A. (2021). Introgression contributes to distribution of structural variations in cattle. *Genomics, 113*(5), 3092-3102. https://doi.org/10.1016/j.ygeno.2021.07.005

Warner, R. D. (2017). The Eating Quality of Meat - IV Water-Holding Capacity and Juiciness. In F. Toldrá (Ed.), *Lawrie's Meat Science* (8th ed., p. 419-459). Woodhead Publishing. https://doi.org/10.1016/B978-0-08-100694-8.00014-5

Weglarz, A. (2010). Meat quality defined based on pH and colour depending on cattle category and slaughter season. *Czech Journal of Animal Science, 55*(12), 548-556. https://doi.org/10.17221/2520-CJAS

Xin, J., Zhang, L., Li, Z., Liu, X., Jin, H., & Yan, C. (2011). Association of polymorphisms in the Calpain I gene with meat quality traits in Yanbian Yellow cattle of China. *Asian-Australasian Journal of Animal Sciences, 24*(1), 9-16. https://doi.org/10.5713/ajas.2011.90407

Zalewska, M., Puppel, K., & Sakowski, T. (2021). Associations between gene polymorphisms and selected meat traits in cattle - A review. *Animal Bioscience, 34*(9), 1425-1438. https://doi.org/10.5713/ab.20.0672