Journal of Agricultural Science and Technology A 9 (2019) 364-370
doi: 10.17265/2161-6256/2019.06.005

Polymorphisms of the CAPN1, CAST and MSTN Genes, and Their Association with Histological and Textural Parameters of Beef from Polish Holstein-Friesian Cattle

Węglarz Andrzej[1], Balakowska Anna[2] and Kułaj Dominika[3]

1. Department of Genetics, Animal Breeding and Ethology, University of Agriculture in Krakow, al. Mickiewicza 24/28, Krakow 30-059, Poland

2. Technology Transfer Center, University of Agriculture in Krakow, al. Mickiewicza 21, Krakow 31-120, Poland

3. Department of Animal Reproduction, Anatomy and Genomics, University of Agriculture in Krakow, al. Mickiewicza 24/28, Krakow 30-059, Poland

Abstract: The purpose of this study was to analyse polymorphisms of the CAPN1, CAST and MSTN genes and their association with the microstructure of the Musculus longissimus thoracis (MLT) and textural parameters in bulls of the Holstein-Friesian breeds, black-and-white variety. The polymorphisms at the three loci: in position 6536 of the 3'UTR region of the CAPN1 gene, in position 230 of intron 5 in CAST gene, and in position 371 of the promoter region of the MSTN gene were analysed. Given the inconsequential genetic diversity at the analysed CAPN1 and MSTN loci in the animal sample, it was considered unreasonable to perform further statistical analyses aimed at determining associations between polymorphisms in these positions and meat characteristics. Based on an analysis of the CAST gene polymorphism, a significant association with certain histological and textural parameters was identified.

Key words: Gene polymorphism, beef, histology, texture, calpain, myostatin.

1. Introduction

Cattle parameters that are of significance in economic terms, including the carcass classification score, can be improved based on genetic assessment or by estimating the breeding value based on phenotype and origin of parents [1]. Therefore, it is necessary to expand the understanding of genes and chromosome regions associated with the desirable parameters. Their verification will contribute to genetic advancements in the cattle breeding sector [2].

Of predominant relevance to the meat tenderisation process are enzymes, endogenous sarcoplasmic proteases, calpains and their inhibitory enzyme calpastatin. Meat hardness is associated with the activity of calpains. The higher the calpain/calpastatin ratio, the greater the meat tenderness [3, 4].

Besides the calpain- and calpastatin-coding genes, a number of studies point to the association between meat quality parameters and the myostatin-coding gene. The association between the MSTN gene and meat tenderness is not as clear as that of calpain and calpastatin. However, myostatin participates in the differentiation of muscle fibres during their formation, and as a result polymorphisms of this gene can lead to different thickness of muscle fibres and different populations of red, white and intermediate fibres in muscles. Thickness of muscle fibres varies according to their type (red fibres have greater thickness than white fibres), and fibres with smaller diameter contribute to greater meat tenderness. Therefore, of relevance to the tenderisation process is the percentage of the specific types of muscle fibres, and as a result, the MSTN gene can be considered as one

Corresponding author: Węglarz Andrzej, Ph.D., research fields: genetics and cattle breeding, beef quality.

of the determinants of meat tenderness [5].

A number of studies have demonstrated that various polymorphisms of the CAPN1, CAST and MSTN genes are associated with meat performance characteristics in numerous cattle breeds. So far, however, few studies in this area concerned cattle reared in Poland, including the Holstein-Friesian breed. Moreover, not all of the studies have included an analysis of significant parameters which are of relevance to the quality of beef [6].

Given the above, the purpose of this study was to analyse polymorphisms in position 6536 of the 3'UTR of the CAPN1 gene, in position 230 of intron 5 in the CAST gene, and in position 371 of the promoter region of the MSTN gene, and to analyse their association with the microstructure of the Musculus longissimus thoracis (MLT) and textural parameters.

2. Materials and Methods

2.1 Animals

The study included 69 bulls of the Polish Holstein-Friesian breed, black-and-white variety. The animals originated from one cattle farm. Bulls were kept under similar environmental conditions and reared on a semi-intensive beef production system. They were slaughtered after reaching the age of 25 months and their average body weight at that point in time was about 615 kg. The slaughter was carried out in accordance with applicable standards in the European Union. Following the slaughter, the carcasses were classified in accordance with the EUROP beef carcass classification system. The conformation of carcasses of bulls was graded P+ to O+. In terms of fat cover, carcasses of animals were graded into fat classes 1 to 3. Then, the carcasses were cooled (24 h), the longissimus thoracis muscles were cut between the 11th and 13th thoracic vertebra, and next were packed in vacuum and matured (10 d).

2.2 Texture Profile Analysis (TPA)

After maturation period, meat samples of about 300 g, wrapped in aluminium foil, were subjected to heat treatment in an electric oven at a temperature of 165 °C until the internal temperature of muscles reached 78 °C. Following the heat treatment and cooling on ice, the samples were cut along the muscle fibres so as to obtain cylinders of diameter 16 mm and height 15 mm, which were used for instrumental TPA. The analysis was performed using a TA-XT2 texture analyser (Stable Micro Systems, Vienna Cort, Lampas Road, Godalming, Surrey GU7 1JG, UK) with Warner-Bratzler attachment featuring a triangular slot blade. The TPA was performed using the same texture analyser with attachment in the form of a cylinder of 50 mm in diameter. The samples were subjected to a 2-squeeze test so as to achieve up to 70% deformation of their height. The travel speed of the cylinder was 2 mm/s, the interval between squeezes—3 s, and the minimum detectable sample size—10 g. The TPA test mimics the mouth's biting action (compression) of the person testing a meat sample. The TPA analysis included primary textural parameters such as hardness, cohesiveness, springiness, resilience and adhesiveness, and secondary textural attributes such as chewiness which is related to hardness, cohesiveness and springiness.

2.3 Microstructure Analysis

The assessment of microstructure involved the determination of thickness and the percentage of specific types of muscle fibres. Specimens for further examination were prepared using meat samples frozen in liquid nitrogen and then kept at a temperature of -80 °C. The frozen material was attached to holders using OCT Embedding Matrix (CellPath Ltd., Newtown, UK) for cryo-specimens. Next, the biological material was cut into sections of thickness 10 μm in a Slee MEV cryostat (SLEE Medical GmbH, Mainz, Germany) at a temperature -20 °C. The resulting muscle sections were fixed onto Superfrost slides (Gerhard Menzel, Glasbearbeitungswerk GmbH & Co. KG, Braunschweig, Germany) coated with

poly-L-lysine. The specimens were subjected to double histochemical and immunohistochemical reaction in accordance with the method developed by Wojtysiak and Kaczor [7]. The specimens were analysed using a Nikon E600 optical microscope (Nikon Instech Co. Ltd. Kawasaki, Japan). The measurements were performed using MultiScan v.14.02 software. The diameter of muscle fibres was determined based on results of measurements carried out for 100 randomly chosen fibres of each type. The percentage of the individual types of muscle fibres was estimated based on the average population of the respective fibres in 10 randomly chosen muscle bundles.

2.4 Analysis of Polymorphisms in the CAST, CAPN1 and MSTN Genes

Segments containing intron 5 of the CAST gene, the 3'UTR region of the CAPN1 gene and the promoter region of the MSTN gene were replicated by polymerase chain reaction (PCR) using a C-1000 thermocycler (BioRad, Carlsbad, United States). The PCR for each segment was performed in a final volume of 20 μL, containing approximately 100 ng template DNA, 2 μL 10× PCR buffer (in case of CAPN1 gene-HotStart PCR buffer), 2.25 mM $MgCl_2$, 0.21 mM dNTPs, 0.35 μM of each primer, and 0.875 U/μL of Taq polymerase (in case of CAPN1 gene-Taq HotStart polymerase). For the CAST gene, the primers were used [8]. This pair of primers was designed based on a partial genomic DNA sequence of the bovine CAST gene (accession number AY008267). The amplification was carried out under the following thermal cycling conditions: initial denaturation at 95 °C for 3.5 min, followed by 36 cycles of denaturation at 94 °C for 45 s, annealing at 69 °C for 45 s, elongation at 72 °C for 1.5 min, and final elongation at 72 °C for 6 min. The primers used for the analysis of the CAPN1 genes were designed by Juszczuk-Kubiak *et al.* [9]. In this case, the thermal cycling conditions were as follows: initial denaturation at 95 °C for 15 min, followed by 33 cycles of denaturation at 94 °C for 60 s, annealing at 59 °C for 30 s, elongation at 72 °C for 40 s, and final elongation at 72 °C for 10 min. The polymorphism of the MSTN gene was analysed using the 5'-CTGAGGGAAAAGCATATCAAC-3' and 5'-TAAAACCCTGTCTGTCACAAG-3' primers which had been designed using Primer3plus software (www.bioinformatics.nl/primer3plus), under the following thermal cycling conditions: initial denaturation at 95 °C for 3.5 min, followed by 36 cycles of denaturation at 94 °C for 45 s, annealing at 55 °C for 45 s, elongation at 72 °C for 1.5 min, and final elongation at 72 °C for 6 min.

To identify polymorphic sites in the gene fragments under investigation, PCR products—14 randomly chosen samples—were subjected to Sanger sequencing. Both the preparations of libraries and the sequencing of PCR products were carried out by Genomed joint stock company. The sequencing reaction was performed using a BigDye® Terminator v3.1 kit (Applied Biosystems, Life Technologies, Carlsbad, United States). Products of the sequencing reaction were separated using the capillary array of a 3730xl DNA Analyser (Life Technologies, Carlsbad, United States).

The amplified products were digested with appropriate restriction enzymes (MboII in case of CAPN1 gene, RsaI in case of CAST gene and DraI in case of MSTN gene).

The amplicons were digested in a reaction mixture containing 10 μL PCR products, 2 μL of 1× buffer 1.05 μL (10.5 U/μL) of appropriate restriction enzyme (Life Technologies, Carlsbad, United States) and 10.5 μL of ultra-pure water. The reaction mixture was incubated on a heating block at a temperature of 37 °C for 90 min. The digestion of the PCR products was stopped by adding 2.5 μL of the loading buffer (10 mM Tris-HCl (pH 7.6), 0.03% bromophenol blue, 0.03% xylene cyanol FF, 60% glycerol, 60 mM ethylenediaminetetraacetic acid (EDTA)). Restriction

fragment length polymorphism (RFLP) products were analysed using electrophoresis in 3.5% agarose gel with an admixture of ethidium bromide and in the presence of pUC19 DNA/MspI Marker (Life Technologies, Carlsbad, United States). Electrophoresis was run for 30 min at 100 V.

2.5 Statistical Analysis

The association between the frequency of specific genotypes and the specific meat parameters was examined based on the Kruskal-Wallis test. Calculations were performed using SAS analytics software (SAS® 9.2, licence number: 9BM1KV).

3. Results and Discussion

It was demonstrated the presence of two alleles (C, T) and two genotypes (*CC*, *CT*) in the CAPN1 locus. In the analysed cattle group the *TT* genotype was no found. The allelic and genotype frequencies at the CAPN1 locus are summarised in Table 1. An analysis of the polymorphism in position 230 of intron 5 in the CAST gene showed two alleles: G and C, and all the three possible genotypes. The *GG* genotype was observed to be most frequent (0.464) in the analysed cattle group. The PCR-RFLP analysis demonstrated the presence of two alleles and two genotypes in position 371 of the promoter region of the myostatin gene. Most animals had the *TT* genotype and none of the analysed bull had the *AA* genotype.

Due to the low frequency of the *CT* genotype in the analysed cattle group, it was considered unreasonable to perform further statistical analyses aimed at determining the association between the genotype at the CAPN1 locus and the meat characteristics under investigation.

The results of the longissimus dorsi histological assessment, as summarised in Table 2, show differences in the population of fibre types between the individual genotypes. The greatest population of fibres was observed for the *CC* genotype and the lowest for the *GG* genotype, however, these differences were not supported statistically. On the other hand, an analysis of the percentage of intermediate fibres showed significant differences between the *GG* and *CC* genotypes within the analysed population. An analysis of the fibre diameter results showed that in analysed animals, all the three types of fibres had greater thickness in bulls of the *GC* genotype as compared to those of the other genotypes. However, none of the differences were found to be significant.

The results of the longissimus thoracis texture assessment are summarised in Table 3. The differences between the genotypes of bulls of the analysed population, though identified in the context of meat parameters such as hardness, adhesiveness, springiness, cohesiveness, gumminess, chewiness and resistance, were not supported statistically. It was found that significance was only the differences between the *GC* and *CC* genotypes in terms of resistance to cutting, which might imply that the *GG* genotype is associated with greater meat tenderness.

It was found that only one animal in the analysed population was heterozygous in position 371 of the promoter region of the myostatin gene, and the other animals were of the *TT* genotype. Given the inconsequential genetic diversity at the analysed locus in the animal sample, it was considered unreasonable

Table 1 The allele and genotype frequencies of analysed gene loci (CAPN1, CAST, MSTN).

Gene	Genotype frequencies						Allele frequencies	
CAPN1	*CC*	*n*	*CT*	*n*	*TT*	*n*	C	T
	0.928	64	0.072	5	0	0	0.964	0.036
CAST	*GG*	*n*	*GC*	*n*	*CC*	*n*	G	C
	0.464	32	0.449	31	0.087	6	0.688	0.312
MSTN	*TT*	*n*	*TA*	*n*	*AA*	*n*	T	A
	0.986	68	0.014	1	0	0	0.993	0.007

n: number of animals.

Table 2 The average results of the longissimus thoracis histological assessment for bulls of different genotypes in position 230 of intron 5 of the CAST gene.

Item	Genotypes		
	GG	*GC*	*CC*
	n = 32	*n* = 31	*n* = 6
Number of total fibres	82.96 (± 28.15)	84.56 (± 24.19)	100.30 (± 14.42)
Number of red fibres (I)	29.21 (± 12.12)	29.13 (± 11.05)	30.80 (± 6.44)
Number of intermediates fibres (II A)	15.66 (± 6.72)	14.09 (± 4.99)	14.28 (± 3.01)
Number of white fibres (II B)	38.14 (± 18.14)	41.34 (± 13.88)	55.21 (± 8.10)
Percentage of fibre type (%)			
Type I	44.32^{A} (± 0.14)	48.33 (± 0.08)	55.03^{A} (± 0.04)
Type II A	20.29^{a} (± 0.10)	17.59 (± 0.08)	14.33^{a} (± 0.03)
Type II B	35.38 (± 0.09)	34.10 (± 0.07)	30.67 (± 0.04)
Diameter of fibre (μm)			
Type I	58.79 (± 6.51)	61.64 (± 9.14)	57.30 (± 11.05)
Type II A	50.93 (± 5.60)	53.19 (± 6.56)	49.14 (± 10.12)
Type II B	46.10 (± 6.48)	47.25 (± 6.75)	42.75 (± 9.10)

Results in lines marked with the same letters show significant differences, a: $p < 0.05$; A: $p < 0.01$.

Table 3 The average results of the longissimus thoracis texture assessment for bulls of different genotypes in position 230 of intron 5 of the CAST gene.

Item	Genotypes		
	GG	*GC*	*CC*
	n = 32	*n* = 31	*n* = 6
Hardness (N)	9.25 (± 5.71)	7.23 (± 2.75)	8.12 (± 2.07)
Adhesiveness (N × s)	-1.29 (± 1.15)	-1.60 (± 1.85)	-1.41 (± 1.05)
Springiness (-)	0.46 (± 0.04)	0.45 (± 0.05)	0.48 (± 0.04)
Cohesiveness (-)	0.55 (± 0.06)	0.56 (± 0.03)	0.55 (± 0.05)
Gumminess (-)	4.96 (± 2.88)	4.03 (± 1.49)	4.35 (± 1.10)
Chewiness (N)	2.31 (± 1.34)	1.85 (± 0.60)	2.07 (± 0.56)
Resilience (-)	0.24 (± 0.02)	0.24 (± 0.02)	0.23 (± 0.02)
Shear force (kg/cm^2)	4.15^{a} (± 1.48)	5.15^{a} (± 2.04)	4.71 (± 1.69)

Results in lines marked with the same letters show significant differences, a: $p < 0.05$.

to perform further statistical analyses aimed at determining the association of polymorphisms in this position and meat characteristics under investigation.

The CAPN1 locus tests were performed for various cattle breeds by a number of authors and they involved different polymorphic sites, identified both in the coding and non-coding regions [9]. The polymorphism in position 6536 of the 3'UTR region was studied [10], and the resulting allelic and genotype frequencies for Holstein-Friesian cattle were different from results of the present studies. These authors showed that nearly half of the animals under investigation were heterozygous at the analysed locus. Other studies support the high frequency of the *CT* genotype (0.58) within Holstein-Friesian cattle [6]. The same tests carried out for meat-producing breeds showed that the *CT* genotype was also most frequent—it was identified in about 56% of animals of the Limousine breed and 65% of animals of the Charolaise breed. The exception was the Hereford breed which was characterised by the dominance of *CC*-homozygous animals (0.538). According to these authors, an equally high frequency of the *CC* genotype was determined in 60% of the analysed animals of the Polish red breed.

Results published by numerous authors indicate that allelic frequencies in relation to the polymorphic site under investigation varied to a great extent according to cattle breed. In most cattle breeds, the C allele was more frequent than the G allele, except the Simmental [8], Aosta Black Pied [11].

Results of the present studies support those obtained by other authors who demonstrated that the A allele frequency was low in a number of analysed cattle breeds [12].

In researches of Iwanowska *et al*. [13] the changes in the tenderness score following varying intervals after the slaughter (48, 96 and 240 h), taking into account the genotype were determined. Based on an analysis of the shear force, it was determined that animals with two C alleles were characterised by the lowest meat tenderness. It was also found that the *CT* and *TT* genotypes have statistically significant association with meat tenderness in terms of the tenderness score after 240 h following the slaughter. Results presented by Juszczuk-Kubiak *et al.* [10] indicate the absence of any statistically significant association between the genotype and parameters assessed after 48 h following the slaughter, such as pH, fat content, weight loss during heat treatment, water retention capacity, tenderness in terms of Warner-Bratzler shear force, and the results of measurements performed after 72 h following the slaughter to assess parameters such as succulence, colour, aroma and smell.

In studies of Avilés *et al*. [14] statistically significant differences in the shear force for raw meat from Limousin cows were demonstrated. Results obtained for animals of the *GC* genotype were significantly different from that obtained for animals of the *CC* genotype. No significant differences were observed for the *GG* genotype. No genotype-related associations were determined based on measurements performed for cooked meat. The association between the *CC* genotype and the higher meat tenderness score in Nellore cattle was also confirmed by Pinto *et al.* [15].

In studied on European beef cattle breeds by measuring the shear force after 2, 7, 14 and 21 d of maturation [8], they found significant differences in the shear force after 2 d and 7 d of maturation for the *CC* and the *GG* genotypes. Moreover, similar significant differences were identified on day 21 for the *CC* and the *GC* genotypes. An analysis of the measurement results at different points in time during meat maturation periods showed significant differences between *CC*, *GC* and *GG* genotypes. It was also determined that in all the analysed meat maturation periods, the presence of the C allele was associated with the reduced percentage of hard steaks.

Results of the present studies show that the *GG* genotype was associated with the highest meat tenderness score and confirm the results of other authors regarding the impact the *CC* genotype on improving meat tenderness.

The existing literature concerning the analysed MSTN polymorphism contains few studies describing associations with the performance characteristics of cattle meat. The research results indicate that the *AA* genotype had a statistically significant association with the higher meat quality grade index and the fat colour index [13]. However, none of the genotypes was found to be associated with the slaughter weight, carcass weight, backfat thickness, loin eye size, marbling score and meat colour index. The results obtained for the Holstein-Friesian and the Japanese black support that there is no association between the genotype and the analysed meat characteristics.

In researches demonstrated that the *TA*-heterozygous Qinchuan cattle was characterised by a significantly higher body length index as compared to animals of the *AA* genotype [16]. The genotype was found to be of no relevance to the body size within the Jiaxin breed. However, within the Nanyang breed, 6-month-old animals of the *AA* genotype were characterised by significantly reduced heart girth, heart girth index and the ratio of heart girth and body length. The withers height of 18-month-old animals of this breed was observed to be significantly greater.

4. Conclusions

Based on the results obtained, it can be concluded that the genetic diversity at the analysed CAST locus in position 230 of intron 5 implies association with certain beef quality parameters. However, it should be noted that meat quality parameters are determined by numerous genes and multiple environmental factors. Hence, it seems reasonable to continue studies into the association between genetic polymorphisms and meat performance characteristics within a greater population of animals, and to use any identified association for cattle selection purposes in the future.

References

[1] Watanabe, T., Matsuda, H., Arakawa, A., Yamada, T., Iwaisaki, H., Nishimura, S., and Sugimoto, Y. 2014. "Estimation of Variance Components for Carcass Traits in Japanese Black Cattle Using 50K SNP Genotype Data." *Animal Science Journal* 85: 1-7.

[2] Gao, Y., Zhang, R., Hu, X., and Li, N. 2007. "Application of Genomic Technologies to the Improvement of Meat Quality of Farm Animals." *Meat Science* 77: 36-45.

[3] Koohmaraie, M., and Geesink, G. H. 2006. "Contribution of Postmortem Muscle Biochemistry to the Delivery of Consistent Meat Quality with Particular Focus on the Calpain System." *Meat Science* 74: 34-43.

[4] Li, J., Zhang, L. P., Gan, Q. F., Li, J. Y., Gao, H. J., Yuan, Z. R., Gao, X., Chen, J. B., and Xu, S. Z. 2010. "Association of CAST Gene Polymorphisms with Carcass and Meat Quality Traits in Chinese Commercial Cattle Herds." *Asian-Australian Journal of Animal Science* 23 (11): 1405-11.

[5] Branciari, R., Ceccobelli, S., Di Lorenzo, P., Ranucci, D., Miraglia, D., Codini, M., Ceccarini, M. R., and Lasagna, E. 2014. "Characterization of Muscle Fibers in Normal and Hypertrophied Marchigiana Beef Cattle." *Journal of Biotechnology* 185, S42.

[6] Juszczuk-Kubiak, E., Flisikowski, K., and Wicińska, K. 2010. "A New SNP in the 3'UTR Region of the Bovine Calpain Small Subunit (CAPN1S) Gene." *Molecular Biology Reports* 37: 473-6.

[7] Wojtysiak, D., and Kaczor, U. 2011. "Effect of g.2728GNA and g.3996TNC Polymorphisms at the Leptin Gene Locus on Microstructure and Physicochemical Properties of Longissimus Lumborum Muscle of Polish Landrace Pigs." *Folia Biologica* 59: 77-82.

[8] Schenkel, F. S., Miller, S. P., Jiang, Z., Mandell, I. B., Ye, X., Li, H., and 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: 291-9.

[9] Morris, C. A., Cullen, N. G., Hickey, S. M., Dobbie, P. M., Veenvliet, B. A., Manley, T. R., Pitchford, W. S., Kruk, Z. A., Bottema, C. D. K., and Wilson, T. 2006. "Genotypic Effects of Calpain 1 and Calpastatin on the Tenderness of Cooked M. Longissimus Dorsi Steaks from Jersey × Limousin, Angus and Hereford-Cross Cattle." *Animal Genetics* 37 (4): 411-4.

[10] Juszczuk-Kubiak, E., Rosochacki, S. J., Wicińska, K., Słoniewski, K., Łyczyński, A., and Sakowski, T. 2007. "Association of the SNP in the 3'-UTR Region of the Small Subunit of Bovine Calcium-Activated Neutral Protease (CAPN1S) with the Activity of the Calpain II and Meat Quality Traits of Fresian Bulls." *Eurobiotech* 4: 25-7.

[11] Lisa, C. D., and Stasio, L. 2009. "Variability of μ-Calpain and Calpastatin Genes in Cattle." *Italian Journal of Animal Science* 8: 99-101.

[12] Han, S. H., Cho, I. C., Ko, M. S., Kim, E. Y., Park, S. P., Lee, S. S., and Oh, H. S. 2012. "A Promoter Polymorphism of MSTN g.-371T > A and Its Associations with Carcass Traits in Korean Cattle." *Molecular Biology Reports* 39: 3767-72.

[13] Iwanowska, A., Grześ, B., Mikołajczak, B., Iwańska, E., Juszczuk-Kubiak, E., Rosochacki, S. J., and Pośpiech, E. 2011. "Impact of Polymorphism of the Regulatory Subunit of the μ-Calpain (CAPN1S) on the Proteolysis Process and Meat Tenderness of Young Bulls." *Molecular Biology Reports* 38: 1295-300.

[14] Avilés, C., Juárez, M., Peña, F., Domenech, V., Clemente, I., and 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: 479-87.

[15] Pinto, L. F. B., Ferraz, J. B. S., Meirelles, F. V., Eler, J. P., Rezende, F. M., Carvalho, M. E., Almeida, H. B., and Silva, R. C. G. 2010. "Association of SNPs on CAPN1 and CAST Genes with Tenderness in Nellore Cattle." *Genetics and Molecular Research* 9: 1431-42.

[16] Zhang, R. F., Chen, H., Lei, C. Z., Zhang, C. L., Lan, X. Y., Zhang, Y. D., Zhang, H. J., Bao, B., Niu, H., and Wang, X. Z. 2007. "Association between Polymorphisms of MSTN and MYF5 Genes and Growth Traits in Three Chinese Cattle Breeds." *Asian-Australian Journal of Animal Science* 20: 1798-804.