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The effect of polymorphisms in the intron 12 of *CAST* gene on meat quality of young bulls*

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The single and combined effects of polymorphism in the intron 12 of the bovine calpastatin (*CAST*) gene on quality traits of meat were examined in seventy-one young bulls of four beef breeds (Charolaise, Limousine, Aberdeen Angus, Hereford) and one dual purpose breed (Simmental). Three single nucleotide polymorphisms (SNP) were considered. The analysed meat traits were pH, fat content, water-holding capacity, thermal drip loss, and results of instrumental and sensory evaluation, all measured in *longissimus dorsi* (LD) muscle 48 or 72 h *post mortem*. The genetic variants at the intron 12 of the bovine *CAST* gene showed a marked effect on beef quality. Analyses of meat revealed differences between animals of different genotypes. Genotypes TC/TA at *locus CAST/Alu*I-*Bse*YI and GG at *locus CAST/Nde*I showed lower thermal drip loss than two remaining genotypes at each of these *loci.* Moreover, allele G at the polymorphic *Nde*I site appeared favourable for the tenderness of beef as evaluated by penetration force. In addition, significant relations were shown between combined *CAST* genotypes and tenderness, juiciness and flavour of meat.

KEY WORDS: beef / *CAST* / cattle / gene polymorphism /meat quality

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Tenderness is one of the most important factors leading to consumer satisfaction when eating beef. In skeletal muscle cytoplasm there are enzymes that are responsible for *post mortem* proteolysis during cold storage of meat. This process is mostly influenced by the μ- calpain (CAPN1) and m-calpain (CAPN2), encoded by the *CAPN1* and *CAPN2* genes, respectively. Calpastatin (CAST) is an endogenous calpain-specific inhibitor, encoded by the *CAST* gene, inhibiting the calpain activity in tissues *post mortem,* and thus regulating the rate and extent of tenderization [Koohmaraie 1996]. Increased *post mortem* CAST activity has been correlated with reduced meat tenderness [Pringle *et al.* 1997]. The bovine *CAST* gene has been mapped to BTA7, with relative position of 117.8 cM [Kappes *et al*. 1997]. Sequencing the bovine calpastatin gene has shown that the gene contains 35 exons in 130 kb of sequence, including 5 exons upstream from exon 2.

Calpastatin exists in several isoforms, the predominant skeletal muscle form, however, consists of four repetitive calpain-inhibiting domains in series with an N-terminal leading region [Raynaud *et al.* 2005]. The variation in the *CAST locus* of cattle has been studied by numerous authors. Bishop *et al.* [1993] found RFLP polymorphism of the bovine *CAST locus* using *Taq*I, *Bam*HI and *Eco*RI endonucleases. This DNA polymorphism was detected in the region encoding domains 2 through 4 plus 3`UTR. Chung *et al*. [1999] noticed genetic variation in the coding for region of domains L and I. They identified three different SSCP patterns in the 1C/1D region of the bovine *CAST* gene. However, Killefer and Koohmaraie [1994] did not detect the polymorphism using as a probe generated fragment from cDNA coding for domains L and I of bovine *CAST*. Lonergan *et al*. [1995] reported of *Bam*HI and *Eco*RI PCR-RFLP using a 2.2 kb cDNA probe coding for domains 2, 3, 4 and 3` UTR of *CAST* gene. The amplified regions of the *CAST* gene were not analyzed by sequencing and these authors did not show the exact location and the kind of these mutations. Nonneman *et al*. [1999] found polymorphism in the microsatellite $(CA)_n$ within promoter region of the *CAST* located 1.4 kb upstream of the transcription start site. Chung *at al*. [2001] found DNA polymorphism in the intron 6 using PCR-RFLP technique and *Xmn*I as the restriction enzyme. Two other SNPs being a transversion G/C in intron 5 (RFLP/*Rsa*I) and transition A/G in the 3'UTR region of the gene were identified by Barendse [2002]. These SNPs are currently used as the IGENITY *Tender*Gene marker and Gene-STAR tests, respectively, as they have been shown to be associated with meat tenderness in beef cattle (Schenkel *et al.* 2006). In the earlier authors' study [Juszczuk-Kubiak *et al*. 2007] using the PCR-SSCP method four SNPs in intron 12 were identified, what was confirmed by the RFLP for three SNPs.

The objective of this study was to examine the effect of polymorphism in the intron 12 of the bovine *CAST* gene on the traits related to meat quality. Three different single nucleotide polymorphisms (SNPs) were analysed. For that, the effects of the combined *CAST* genotypes were estimated. The traits analysed were physico-chemical parameters of beef and its sensory evaluation score.

Material and methods

Animals

The study was performed on 71 bulls of five breeds – Charolaise, Limousine, Aberdeen Angus, Hereford and Simmental (n=18, 16, 10, 16, 11, respectively). The bulls of each breed were the randomly chosen progeny of 5-7 sires. At the age of 6-7 months, the bulls were transferred to the Institute farm, Jastrzębiec. After reaching the age of 9 months the bulls were fed ad libitum until the 15th month of life with total mixed ration (TMR), consisted of corn silage (65% of dry matter), hay (5% DM) and concentrate (30% DM). One kilogram of TMR dry matter contained 106 g crude protein, 71 g microbial protein supplied from rumen-degraded protein (PDIN), 93 g microbial protein supplied from rumen-fermented organic matter (PDIE) and 90 Feed Unit for maintenance and meat production (UFV). PDIN consisted of protein digested in the small intestine, which is not digestible in rumen forage protein (PDIA) and protein digested in the small intestine (PDI) [Jarrige 1989]. The bulls were weighed monthly, then weighed at the age of 15 months and slaughtered in the local abattoir. All procedures involving animals were approved by a Local Ethics Commission for Experimentation on Animals (permission No. 67/2001).

Analytical

From right carcass-sides samples of *Longissimus dorsi* (LD) muscle (approximately 900 g) were excised one hour post slaughter, covered with comminuted ice and brought to the Department of Meat Technology, Warsaw Agricultural University. Analyses of meat samples were performed after storage in cold room at 4±2şC, for 48 hours.

The tenderness of meat was determined by penetration force (PF_{48}) and shear force ($SF4_{48}$) test. The slice of meat of about 150 g and 3 cm thick heated for 2 h at 78°C in 1% solution of table salt and then chilled for 24h at 4°C. The penetration force (PF_{48}) meat was measured using ZWICK type 1120 (Zwick, Ulm, Germany), crosswise the muscle fibres of meat sample (20x40x15 mm) with a plunger of 13 mm diameter. The velocity of the plunger was 50 mm/min. The force of penetration (PF_{48}) was measured after the gauge plunger, which was moved up to 10 mm depth after the initial tension of the fibres appeared. The measurement of the shear force was conducted with a use of shear element of Warner Bratzler type (model 4201, Inston Corporation, USA). The maximum shear force was read by move of 50 mm/min. The meat probes 20x40x20 mm large were investigated. The shear force was tested against muscle fibres.

The drip loss (DL_{48}) was estimated on 30 g of minced meat placed in a glass beaker for 30 minutes of heating at 70±2°C in water bath. After heating, the sample of meat was chilled at room temperature for 30 min. The liquid fraction was removed (outflow) and the beaker was weighted. The loss of weight was estimated as per cent, according to equation:

$$W_t = [(a-b/(a-c)] \times 100\%$$

where:

W_t – the outflow after thermal treatment (%);
a – the weight of meat and the beaker;
b – the weight of beaker with meat after thermal outflow;
c – the weight of empty beaker.

Water-holding capacity (WHC_{48}) was determined with a filter paper method according to Grau and Hamm [1952].

Fat content (Soxhlet method) was determined according to Polish Standard PN-73/A-82111.

In LD muscle homogenates, the pH_{48} was measured 48 h post-slaughter using the CP-315 pH-meter with a combined glass-calomel electrode, according to Polish Standard PN-77/A-82058.

Sensory evaluation

Sensory evaluation was carried out according to a 5-point scale. Colour, taste, flavour and juiciness of meat were evaluated as prescribed by Baryłko-Pikielna *et al*. [1964] and Polish Standard PN-ISO6564. In brief, the 150 g sample of meat was kept firstly at 4°C for 72 h and next for four days in a 1% table salt solution. The meat was then fried for 8 min on each side at 180°C. Scoring was performed by a panel of five experienced people.

Determination of *CAST* polymorphism

DNA was isolated from the blood according to Kanai *et al*. [1994]. Two linked mutations RFLP – *Alu*I T/C (3893+155*)/*BseY*I T/A (3893+223*) – and the third one –RFLP/*Nde*I A/G (3893+428*) within intron 12 of the *CAST* gene were identified according to Juszczuk-Kubiak *et al*. [2007].

Statistical

The effects of single and combined genotypes on the traits studied were analysed using the least-squares method as applied in the general linear model (GLM) procedure of SAS, according to the following model:

$$y_{ijklm} = \alpha + R_i + G_j + S_k + O_l + \beta(x_{ijklm} - \overline{x}) + e_{ijklm}$$

where:
y_{ijklm} – trait studied;
α – overall mean;

*Position of the mutation in intron was marked with consideration of a position of the last suitable nucleotide exon in the mRNA sequence.

R_i – fixed effect of breed (i = 1, …, 5);

G_j – fixed effect of j-th genotype of *CAST* gene (j = 1,…, 3) or combined genotype (i=1,…, 9);

S_k – random effect of k-th season at the start of fattening (k = 1, 2; November-April, May-October);

O_l – random effect of l-th sire;

$\beta(x_{ijklm} - \bar{x})$ – fixed regression of the analysed trait on carcass weight;

e_{ijklm} – random error.

Data for all animals were analysed jointly and the effect of a breed has been included in the statistical model. The significance of differences was verified with the Duncan test. The model includes nine combined genotypes that were found in the animal sample. However, only the estimated combined genotypes, found in three or more bulls, are presented.

Results and discussion

Genotype and allele frequencies

Genotype and allele/haplotype frequencies for each SNP or combined genotypes are summarized in Tables 1 and 2.

Table 1. The genotype and haplotype/allele frequencies at *Alu*I/*BseY*I/*Nde*I site in the bovine *CAST* gene

Genotype/ allele	Breed				
	Aberdeen-Angus (n=10)	Charolaise (n=18)	Hereford (n=16)	Limousine (n=16)	Simmental (n=11)
			*RFLP-Alu*I/*BseY*I		
TT/TT	-	5	2	2	2
TC/TA	6	9	8	11	8
CC/AA	4	4	6	3	1
			Haplotype		
TT	0.300	0.528	0.375	0.469	0.545
CA	0.700	0.472	0.625	0.531	0.455
			*RFLP-Nde*I		
AA	3	5	3	3	1
AG	5	11	10	8	8
GG	2	2	3	5	2
			Allele		
A	0.550	0.583	0.500	0.438	0.455
G	0.450	0.417	0.500	0.563	0.545

The overall genotypic frequencies for RFLP *Alu*I/*BseY*I and RFLP/*Nde*I mutations were in accordance with Hardy-Weinberg equilibrium. The nine combined genotypes were observed, what is in agreement with theoretically possible combinations of three

Table 2. The combined genotype/ haplotype frequencies at *Alu*/*BseY*I/*Nde*I site in the bovine *CAST* gene

Combined genotype	Breed				
	Aberdeen- Angus (n=10)	Charolaise (n=18)	Hereford (n=16)	Limousine (n=16)	Simmental (n=11)
CAA/CAA	-	1(0.055)	-	-	-
CAA/CAG	-	1(0.055)	-	-	1(0.091)
CAG/CAG	-	1(0.055)	-	1(0.063)	1(0.091)
TTA/CAA	1(0.100)	2(0.111)	3(0.188)	2(0.125)	2(0.182)
TTA/CAG	3(0.300)	5(0.277)	4(0.250)	5(0.312)	4(0.363)
TTG/CAG	-	1(0.055)	2(0.125)	1(0.063)	1(0.091)
TTA/TTA	2(0.200)	2(0.111)	3(0.187)	3(0.187)	-
TTA/TTG	2(0.200)	3(0.170)	3(0.187)	3(0.187)	2(0.182)
TTG/TTG	2(0.200)	2(0.111)	1(0.063)	1(0.063)	-
	Combined haplotype				
TTA	0.500	0.380	0.500	0.500	0.364
CAG	0.100	0.250	0.375	0.250	0.364
CAA	0.050	0.138	0.003	0.063	0.136
TTG	0.350	0.232	0.122	0.187	0.136

individual genotypes (3^2). The genotypes frequency showed a great predominance of the TTA/CAG genotype in bulls of all examined breeds, however CAA/CAA genotype frequency was found out exclusively in Charolaises (only one animal).

Effect of single genotypes on meat parameters

The individual *CAST* genotype (SNP-*Alu*I/*BseY*I or SNP/*Nde*I) effects on the meat quality parameters are shown in Table 3. The differences were found in meat quality between examined breeds.

The significant influences of SNP-*Alu*I/*BseY*I polymorphisms were observed on WHC_{48}, DL_{48}, and fat content (%). The meat of bulls of TC/TA genotype showed higher (lower value) WHC_{48} ($P \leq 0.05$) and contained less fat ($P \leq 0.01$) compared to animals of TT/TT genotype. DL_{48} was significantly higher in the meat of animals with the CC/AA genotype compared to the meat of TC/TA animals ($P \leq 0.05$).

A significant association was observed of SNP/*Nde*I polymorphism with meat tenderness determined by PF_{48}. The beef of bulls of a genotype GG was more tender than the meat of bulls of AA ($P \leq 0.01$) and AG ($P \leq 0.05$) genotype. Moreover, the meat of bulls of genotype GG showed lower DL_{48} ($P \leq 0.05$) than of bulls with AG variant of *CAST*. The *CAST* genotype had significant effect neither on the LD pH_{48} nor on the score given by sensory evaluation panel ($colour_{72}$, $flavor_{72}$, $taste_{72}$ and $juiciness_{72}$).

Effect of combined genotypes on meat parameters

Some of the combined genotypes appeared to have significant effects on the measured traits. The meat from animals carrying the CAG/CAG combined genotype was more tender ($P \leq 0.05$) than that of animals of remaining combined genotypes (Tab. 4). The meat of the combined bulls' genotype TTA/CAA showed the lowest DL_{48} ($P \leq 0.05$) as compared to the meat of animals with CAA/CAG genotype. The more

Table 3. Least squares means (LSM) and standard errors (SE) for traits evaluated of bulls' meat in relation to mutations RFLP-*Alu*I/*Bse*YI and RFLP/*Nde*I in intron 12 of *CAST* gene

SNP	Genotype		pH_{48}	Fat (%)	WHC (cm^2/)	DL (%)	PF (N)	SF (N/cm^2)	Colour (pts)	Taste (pts)	Flavour (pts)	Juiciness (pts)
Intron 12 *RFLP/Alu*I/*Bse*YI	CC/AA (n=6)	mean	5.52	3.38	28.33	7.82^{a}	59.51	25.27	4.43	4.53	4.52	4.57
		SE	0.02	0.43	1.72	1.15	6.58	3.19	0.11	0.10	0.13	0.12
	TC/TA (n=35)	mean	5.50	2.60^{A}	26.93^{a}	5.62^{a}	67.11	28.96	4.40	4.67	4.63	4.58
		SE	0.03	0.52	1.94	1.12	5.03	2.43	0.13	0.12	0.16	0.12
	TT/TT (n=30)	mean	5.52	3.65^{A}	31.03^{a}	6.81	65.73	26.76	4.48	4.56	4.51	4.57
		SE	0.03	0.55	1.76	1.02	4.33	2.09	0.14	0.13	0.17	0.10
Intron 12 *RFLP/Nde*I	AA (n=20)	mean	5.51	2.90	30.11	6.01	75.95^{Ab}	27.55	4.43	4.56	4.67	4.30
		SE	0.03	0.56	2.22	1.22	5.17	2.67	0.11	0.16	0.13	0.32
	AG (n=35)	mean	5.51	3.26	28.86	7.97^{a}	65.46^{b}	27.85	4.38	4.49	4.58	4.46
		SE	0.03	0.46	1.83	0.99	4.25	2.20	0.11	0.13	0.10	0.13
	GG (n=16)	mean	5.55	3.44	29.80	5.83^{a}	60.31^{A}	26.41	4.48	4.60	4.52	4.47
		SE	0.03	0.49	1.96	1.07	4.75	2.20	0.14	0.14	0.11	0.33

$^{aA\ldots}$Within columns and SNPs means bearing the same superscripts differ significantly at: small letters - $P \leq 0.05$, capitals - $P \leq 0.01$
WHC – water holding capacity, DL – drip loss, PF – penetration force, SF – shear force, n – number of animals.

Table 4. The influence of combined genotypes RFLP/*Alu*I/*BseY*I/*Nde*I in intron 12 of *CAST* gene on some evaluated traits of meat of bulls

SNPs	Genotype (n)	WHC (cm^2/g)	DL (%)	PF (N)	SF (N/cm^2)	Flavour	Juiciness
		LSM±SE	LSM±SE	LSM±SE	LSM±SE	LSM±SE	LSM±SE
intron 12 RFLP-*Alu*I/*BseY*I/*Nde*I	CAA/CAA (1)	ns	ns	ns	ns	ns	Ns
	CAA/CAG (2)	ns	11.48^{ab}±2.6	82.62^{Ab}±6.71	34.89^{a}±3.65	ns	Ns
	TTG/CAG (5)	ns	5.43^{b}±1.49	ns	30.59^{b}±3.20	4.00^{ab}±0.15	Ns
	TTA/CAA (10)	ns	4.9^{a}±1.60	81.58^{B}±6.8	ns	4.34^{b}±0.17	3.80^{ab}±0.26
	TTG/TTG (6)	ns	ns	ns	ns	ns	Ns
	CAG/CAG (3)	ns	ns	48.07^{ABac}±6.8	ns	ns	Ns
	TTA/TTG (13)	ns	ns	66.48^{ab}±6.0	24.62^{ab}±2.6	4.46^{a}±0.12	4.31^{b}±0.19
	TTA/TTA (10)	ns	ns	ns	ns	ns	4.35^{a}±0.21
	TTA/CAG (21)	ns	ns	66.15^{c}±4.8	ns	ns	ns

$^{aA...}$Within columns means bearing the same superscripts differ significantly at: small letters – P≤0.05, capitals – P≤0.01;
WHC – water holding capacity, DL – drip loss, PF – penetration force, CF – shear force; LSM – least squares means, SE – standard errors, ns- not significant, n – number of animals.

intense flavour (P≤0.05) was found out of the meat of bulls with TTA/TTG genotype as compared to the meat of TTG/CAG bulls; also the more desirable juiciness was found out in the meat of TTA/TTA genotype bulls (P≤0.05) as compared to those of the TTA/CAA genotype.

Right now, molecular markers such as the DNA polymorphism, play the key role in the animal genetics. Recently, a number of potential candidate genes for meat quality have been recognized. Allelic variation in the regulatory and structural regions of these genes may affect their expression (what can be seen in the amino acid sequence of a protein) and finally influence the quality traits of beef. Also the variation in the introns or flanking sequences cannot be ignored as the synonymous SNPs in these regions may influence mRNA stability and translation thereby resulting in altered function [Gibbs 2003, Duan *et al.* 2003]. Additionally, the SNP may be in linkage disequilibrium with variation in other regions of the gene with functional or structural significance.

One of such markers, which can be recognized as a good meat quantity determinant, is calpastatin, the endogenous inhibitor of calpains. A number of studies have shown that calpastatin plays a central role in regulation of the calpains activity in the cell and is considered to be one of major modulators of the *post mortem* protein turnover. Therefore, *CAST* may affect proteolysis of myofibrils due to regulation of activity of calpains, and is responsible for initiation of *post mortem* degradation of myofibrillar

protein [Goll *et al.* 2003]. However, the role of this inhibitor in the degradation model of protein is still elucidated. It is known that Calpastatin and calpain activities decline with increasing age of animals. Veiseth *et al.* [2004] showed, that in *longissimus* muscle from lamb ranging 2 to 10 months of age, calpastatin activity decreased, what affected the increase of *post mortem* proteolysis and meat tenderization in older animals.

Starting with Koohmaraie [1994], several associations in sequence variation of calpastatin gene as source of genetic markers, what may influence meat tenderness, have been investigated. The author reported that the genetic correlation coefficient between *post rigor* calpastatin activity and shear force exceeded 0.5. This finding demonstrated that selection against calpastatin activity would correlate with the improvement of meat tenderness. Furthermore, Lonergan *et al*. [1995] using RFLP at the bovine calpastatin *locus* found relationship between calpastatin activity and meat tenderness. Wulf *et al.* [1996] found the residual correlation (0.42) between WBSF (Warner Bratzler shear force) and calpastatin activity in beef breeds, but noticed no correlation between calpastatin activity and meat marbling,

Some earlier data presented and patented by Barendse *et al*. [2002] suggested that genetic variation at the *CAST locus* contributes to variation in meat tenderness traits. Casas *et al.* [2006] reported that SPN located in the 3'UTR region of the *CAST* gene was associated with meat tenderness, juiciness and flavour in *Bos taurus* and *Bos taurus* x *Bos indicus* crossbreds. No such significant association was found in a purebred *Bos indicus* population. The data presented by Casas *et al*. [2006] were the first report on the association of *CAST* SNP with meat tenderness. They proved the potential genetic interaction between markers for two *loci* (*CAPN1* and *CAST*) that are currently being used as the basis of commercial DNA tests for meat tenderness in beef cattle.

Schenkel *et al*. [2006] have shown an association between the polymorphism at the *Rsa*I site in the intron 5 of the *CAST* gene and meat tenderness in beef cattle. Recently Eenennaam *et al.* [2007] have confirmed that meat tenderness could markedly be improved by selection for the favourable *CAST* genotypes of these two mutations [Casas *et al.* 2006, Schenkel *et al.* 2006] that have been included in the GeneSTARTenderness and IGENITY *Tender*GENE marker panels.

Although some suggestive associations between the polymorphism in *CAST* gene and meat tenderness were reported in cattle, none of these studies found any amino-acid sequence variation in the calpastatin protein to be connected with the changes in the potential regulatory regions that could explain phenotypic differences in meat quality. Ciobanu *et al*. [2004] described three mutations located in the region of the *CAST* gene and encoding Domain L, 1 and 4 calpastatin in pigs The newly identified *CAST* variations had significant effects on tenderness and other important pork quality traits.

In the present study, all three polymorphisms studied were SNPs, but two mutations – at position 3893+155* (T/C) and 3893+223* (T/A) – were linked and inherited were as haplotypes. The associations were shown of polymorphism, within

intron 12 of the bovine *CAST* gene at three sites, with traits related to beef quality. The examined *CAST* gene polymorphisms were identified in the non-coding sequence and had no impact on calpastatin structure or function. However, they should not be ignored. Jiang and Gibson [1999] postulated the necessity of estimating the value of mutation of this type for mRNA stability. Additionally, these SNPs may be in linkage disequilibrium with variation in other regions of the gene with functional or structural significance. For example, Kurył *et al.* [2003] reported the influence of *CAST* gene polymorphism in intron 6 on growth and carcass traits in pigs. In the authors' earlier study on Polish Black-and-White cattle and beef bulls similar to these used in the present work, the associations were identified of meat quality with *CAST* genotypes. The polymorphism investigated in exon 1 of *CAST locus* was related to DL_{48} and meat colour, but not to meat tenderness [Juszczuk-Kubiak *et al.* 2004].

In this study, cattle population was used with no family structure. Thus, there was no possibility of studying the segregation of alleles and their effects on traits within a family. For the same reason, identification of true haplotypes was not possible. Instead, the combined genotypes were used to estimate associations with meat production traits. As the analysed variations are in intron, it is rather difficult to conclude about a direct effect of the *CAST* genotypes typed here on meat quality traits involved. Suggested rather may be a linkage to another mutation being a causal mutation in the coding or regulatory regions of the gene. Therefore, the presented results can be interpreted only as a correlation between marker and production, at this time and in this population.

The results of the study presented here can be summarized as follows:

1. The heterozygous genotypes at the polymorphic *Alu*I/*Bse*YI sites appear superior for water-holding capacity and drip loss of beef, both measured 48 h *post mortem*. This suggests the occurrence of molecular positive heterosis, occurring when subjects heterozygous for a specific genetic polymorphism show a significantly greater effect for a quantitative trait than subjects homozygous for either allele.
2. The allele G at the polymorphic *Nde*I site is favourably and significantly associated with better meat tenderness (lower PF_{48}) and significantly lower percentage of drip loss (DL_{48}). This result might suggest a favourable influence of the G allele on the traits investigated.
3. The combined genotype TTA/TTG had significant effects on commercially important beef quality traits such as tenderness and subjective flavour.

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Wpływ polimorfizmu w intronie 12 genu *CAST* na jakość mięsa buhajków

S t r e s z c z e n i e

W przeprowadzonej analizie statystycznej związku polimorfizmu w intronie 12 genu *CAST* z jakością kulinarną i przydatnością technologiczną mięsa wykazano wpływ poszczególnych mutacji na wartość niektórych spośród 10 mierzonych cech jakości wołowiny buhajków czterech ras mięsnych i jednej ogólnoużytkowej. Polimorfizm RFLP-*Alu*I/*Bse*YI w *locus CAST* warunkował istotnie mniejszy ($P<0.05$) wyciek termiczny (DL_{48}), wyższą zdolność utrzymywania wody własnej (WHC_{48}) oraz mniejszą zawartość tłuszczu (%) w mięśniu najdłuższym grzbietu buhajków heterozygotycznych względem analizowanych mutacji. Zależności te obrazują zjawisko wpływu pozytywnej heterozji, a nie wpływu obecności któregoś z alleli. Nie stwierdzono istotnych zależności między polimorfizmem RFLP-*Alu*I/*Bse*YI, a kruchością i walorami sensorycznymi mięsa. Porównując średnie wartości analizowanych cech zwierząt zróżnicowanych genotypem w *locus CAST*/*Nde*I, najkorzystniejsze z punktu widzenia jakości mięsa wartości siły penetracji (PF_{48}) i wycieku termicznego (DL_{48}) odnotowano w mięśniu zwierząt o genotypie GG.

Przeanalizowano również wpływ genotypu względem wszystkich trzech mutacji łącznie u zwierząt o poszczególnych genotypach kombinowanych, odnotowując zależności o tendencji podobnej, jak w przypadku analiz dla obu miejsc polimorficznych ocenianych oddzielnie. Najkorzystniejszą wartość DL_{48} stwierdzono w mięśniu buhajków o genotypie TTA/CAA, natomiast zwierzęta o genotypie TTA/TTG wykazywały najniższe parametry siły cięcia (SF_{48}), w porównaniu ze zwierzętami o innych genotypach. Stwierdzono także istotny wpływ polimorfizmu w intronie 12 w *locus CAST* na parametry oceny sensorycznej mięsa dokonywanej organoleptycznie – zapachu i soczystości. Mięso osobników o genotypie TTA/CAA w zakresie soczystości odznaczało się najmniej korzystną wartością w porównaniu z mięsem zwierząt o genotypie TTA/TTA.