

Influence of calpastatin gene polymorphism (SNP CAST283) on the development of meat qualities of young meat cattle in postnatal ontogenesis

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Abstract. The objective of the study was to investigate the influence of SNP CAST 283 on the development of meat qualities in young cattle of Hereford and Limousin breeds in postnatal ontogenesis. The objectives of the study included: genotyping of young cattle by SNP CAST C283T, determination of genotypic structure, allelic state, level of genetic diversity and equilibrium of the studied herds of Hereford and Limousin cattle, study of live weight dynamics in postnatal ontogenesis, post-slaughter carcass evaluation, chemical composition of beef and structural and mechanical properties of meat obtained from steers of different genotypes. The results of genotyping of bulls of Hereford and Limousin breeds by SNP CAST C283T, testify to high frequency of occurrence in both herds of genotype CAST^{SS} and low desirable genotype CAST^{TT} (7.5% in Herefords and 4.18% in Limousins), a low frequency of the allele CAST^T (0.29 and 0.26) was noted. In our studies, no significant relationship of the studied SNP CAST C283T with weight growth, post-slaughter parameters and chemical composition of meat of steers of different genotypes was found. There was a significant association of the studied polymorphism with lower cutting force of the longest muscle of the back in steers with the genotype CAST^{TT} at the end of the first day and on the third day of the experiment, which allows us to recommend this polymorphism as a marker of "tenderness" of beef in selection work with Hereford and Limousin cattle.

1 Introduction

The CAST gene encodes the most important protein, calpain regulator calpastatin, which inhibits the proteolytic activity of μ - and m calpains, preventing their binding to membranes, thus regulating postmortem proteolysis of meat after slaughter [1, 3, 7, 8, 12, 13]. After cessation of blood circulation, oxygen supply to muscle fibres is impaired. From this point, metabolic processes under anaerobic conditions are triggered in meat, reducing the ATP level in muscle cells. The formation of actomyosin cross-bridges in muscle tissue

under these conditions leads to an increase in meat density. Then comes the stage of natural tenderisation of meat and an increase in its tenderness index [5]. The efficiency of natural tenderisation depends on post-slaughter proteolysis of muscle fibres.

The calpastatin CAST gene consists of 35 exons, has a size of about 149 bp and is located on chromosome 7 [4].

Currently, some polymorphisms of the calpastatin gene are widely used in marker-assisted selection. In test systems GeneSTAR® G/A substitution (base 2959 of AF159246) localised in the 3UTR locus is used. The second commercial test Igenity TenderGENE™ uses a G/C substitution (282 from AY008267) localised in intron 5. In addition, a SNP characterised by an adenine to guanine substitution in g.96165561A > G (rs109221039) is known, the preferred animal genotype is AA, associated with meat "tenderness" [2]. Several studies have reported other genetic variations in the CAST gene occurring in coding regions [6,10,15] and non-coding regions [11] associated with meat tenderness. SNP WSU is a marker representing a cytosine to thymine substitution in exon 3 (base 263 AY008267). Base 263 is much closer to base 283 for UOCAST1, and therefore these markers can be inherited as haplotypes. In Nellore cattle, an additive effect of UOCAST1 was observed at all periods after slaughter and a significant dominance effect at day 7 and 21 of meat maturity [16]. This SNP is based on a C/T substitution at position 283 of the CAST gene sequence (GenBank: NM_174003.2) [9]. In addition, the calpastatin (CAST) gene is also known to be widely expressed in reproductive tissues and organs. An association between SNPs in exon 3 of the CAST gene and fertility in Holstein cattle was found [10, 14]. The use of the developed methods of genodiagnostics of cattle allows the identification of preferred genotypes for the production of more tender meat, because the increase in the frequency of occurrence of preferred alleles of the CAST gene in the population of beef cattle by selection of animals for breeding, contributes to the improvement of organoleptic qualities of meat products.

Thus, the polymorphisms of the capastatin gene associated with certain economically useful traits are now well studied. The most known SNP CAST G282C, which is used to improve the trait "tenderness of meat" in marker-mediated selection of cattle, and little information on SNP CAST C283T located in the same locus.

In this regard, the aim of our study was to investigate the effect of calpastatin gene polymorphism (SNP CAST C283T) on the development of meat qualities in young cattle of Hereford and Limousin breeds in postnatal ontogenesis. The objectives of the study included: genotyping of young cattle by SNP CAST C283T, determination of genotypic structure, allelic state of the gene, level of genetic diversity and equilibrium of the studied herds of Hereford and Limousin cattle, study of live weight dynamics in postnatal ontogenesis, post-slaughter evaluation of carcasses, chemical composition of beef and structural and mechanical properties of meat obtained from steers of different genotypes.

2 Materials and methods

The objects of the research were bulls of Hereford breed in the amount of 80 heads (LLC "SAVA-Argo-Usen") and Limousin breed in the amount of 72 heads ("SAVA-Agro-Yapryk"). During the research period 2019-2020, the farms were breeding farms. Cattle breeding is carried out according to stall and pasture technology with elements of resource conservation.

Genotyping was carried out in the laboratory of DNA-technology of the All-Russian Research Institute of Breeding and in the laboratory of molecular genetics of the Bashkir State Agrarian University. For genotyping, blood samples were taken from the jugular vein of experimental animals into tubes with ethylenediaminetetraacetic acid (EDTA).

DNA extraction was performed using DNA-Extran-1 reagent kit (Syntol Research and

Development Company, Russia) according to the manufacturer's instructions.

A specific fragment 308 bp long in exon 3 of the CAST gene (SNPC283T) was amplified using primers: F: 5'-aaa-ttt-gcg-gtt-gac-cac-act-gtt-a-3'; R: 5'-tgt-tat-gcc-tgt-tgc-ttt-gta-cct-c-3' (Gábor M. et al., 2012) [9]. Gene amplifications were cleaved by MspI endonucleases, respectively. The number and length of restriction fragments were determined electrophoretically in a 3.0% agarose gel in UV light under ethidium bromide staining. Gel Doc XR gel documentation system and Image Lab version 2.0 "DNA-analyser" software were used for gel analysis. Length sizes of restriction fragments: CASTCC - 137,135 bp; CASTCT - 308, 137, 135 bp; CAST^{TT} - 308 bp.

Based on DNA testing data, the frequency of genotype occurrence was determined using the formula: $p = n/N \times 100$ (where p is the frequency of genotype determination; n is the number of individuals with a certain genotype; N is the total number of individuals). The frequency of individual alleles was determined using the following formulas: $pA = (2nAA + nAB)/2N$ (pA is the frequency of allele A) and $qB = (2nBB + nAB)/2N$ (qB is the frequency of allele B).

For comparative assessment of genetic structure, genetic diversity and herd equilibrium, we calculated: H_e defines the expected heterozygosity degree ($H_e = 1 - \sum p_i^2$, where p_i - frequency of occurrence of the i -th allele); the observed degree of heterozygosity ($H_o = n/N$, where n is the number of individuals heterozygous for a given allele, N is the sample size). To assess the correspondence between the actual and expected distribution of genotypes in the studied animal samples, the following χ^2 were used, which was calculated by the formula:

$$\chi^2 = \sum_{k=1}^k (O - E)^2 / E ,$$

where O and E are the observed and theoretically expected numbers of genotypes of a certain type, k is the number of genotypic classes.

Three groups were formed from steers of different genotypes by SNP CAST C283T using the method of live weight analogues: the I experimental group included animals with the genotype CAST^{CC} ($n=20$, Hereford breed; $n=20$, Limousin breed), in II - with the genotype CAST^{CT} ($n=20$ Hereford and $n=20$ Limousin breeds), and in group III - steers with the genotype CAST^{TT} ($n=6$ Hereford and $n=3$ Limousin breeds).

The steers were reared up to 20 months of age by stall-pasture technology with the use of resource-saving elements.

As a lifetime indicator of the development of meat qualities in young animals in postnatal ontogenesis, the change in live weight of steers was studied, which was estimated by the results of control weighing of calves at the age of 8, 12, 16 and 20 months.

Meatiness of cattle was assessed on carcasses of steers of different genotypes in the conditions of meat processing plant "SAVA" according to GOST 33818-2016. Studies of the biochemical composition of the longest muscle of the back were carried out in the analytical laboratory of the Institute of Biological Systems and Agrotechnologies according to GOST 23042-86: Meat and meat products. Methods of fat determination; GOST R 53642-2009: Meat and meat products. Method for determination of mass fraction of total ash; GOST 25011-81: Meat and meat products. Methods for determination of protein. GOST R 51479-99: Meat and meat products. Method for determination of mass fraction of moisture.

Assessment of structural and mechanical properties of the longest muscle of the back of steers of different genotypes of Hereford and Limousin breeds was carried out on 1, 7, 14 and 18 days after slaughter using the Warner-Bratzler device.

The genotype of animals according to SNP CAST C283T was taken into account as a factor influencing the performance of cattle. Arithmetic mean (M) and standard errors of

mean values ($\pm m$) were calculated. Student's t-test was used to identify statistically significant differences in mean values between groups. Statistical processing of the study results was performed using the software application "Excel" from the Microsoft Office package and STATISTICA 5.0.

3 Results

The distribution of genotypes for SNP CAST-C283T among Hereford and Limousin breeds of bull calves is given in Table 1.

Table 1. Distribution of genotypes and allele frequencies for SNP CAST-C283T among Hereford and Limousin bull calves

Genotype	Breed			
	Hereford (n=80)		Limousin (n=72)	
	animal	%	animal	%
Frequency of genotype occurrence				
CC	39	48.75	37	51.38
CT	35	43.75	32	44.44
TT	6	7.50	3	4.18
Allele frequency				
C	0.71		0.74	
T	0.29		0.26	

The distribution of allele frequencies of the CAST SNP C283T gene indicates that the frequency of occurrence of genotype CAST^{CC} in Hereford cattle is lower and is 48.75% compared to 51.38% in Limousin cattle. The frequency of the CAST^{TT} genotype in Hereford cattle was 7.50% and in Limousin cattle 4.18%, which indicates a low occurrence of this allele in the studied herds. The allelic state of the CAST gene by SNP C283T indicates a relatively high occurrence of the allele CAST^C (0.74) in the herd of Limousin cattle.

Indicators of actual and expected heterozygosity are given in Table 2.

Table 2. Indicators of the level of genetic diversity and herd equilibrium

Breed	H _o *	H _e	F	χ ²
Hereford	0.437	0.412	0.025	0.152
Limousin	0.444	0.384	0.060	0.874

*H_o – observed heterozygosity; H_e – expected heterozygosity; F – Ho-He difference («+/-» – heterozygote excess/deficiency), χ² – criterion for matching the observed and expected distribution of genotypes.

The values of observed heterozygosity by SNP CAST C283T slightly exceed the value of theoretical heterozygosity: by 0.025 in Hereford breed and by 0.060 in Limousin breed. We noted the correspondence of the observed and expected distribution of genotypes, which indicates the equilibrium of the studied herds of beef cattle.

The results of studying the dynamics of live weight of steers of different genotypes by SNP CAST C283T of Hereford and Limousin breeds in postnatal ontogenesis are given in Table 3.

Table 3. Change in live weight of steers of different genotypes by SNP CAST C283T of Hereford and Limousin breeds in postnatal ontogeny, kg (M±m)

Age, months	Breed/genotype					
	Hereford (n=46)			Limousin (n=43)		
	CC	CT	TT	CC	CT	TT
Newborns	33.33 ±0.34	33.32 ±0.27	33.13 ±0.68	33.87 ±0.19	34.02 ±0.31	34.00 ±1.16
8	247.68 ±1.25	248.60 ±1.42	253.11 ±2.74	258.57 ±1.76	259.86 ±3.72	265.00 ±2.52
12	357.96 ±2.49	360.08 ±2.02	365.47 ±4.45	375.60 ±2.98	376.5 ±3.71	376.67 ±6.57
16	467.90 ±3.66	471.23 ±3.19	478.44 ±8.05	492.20 ±2.51	492.25 ±2.45	498.67 ±0.89
20	570.06 ±4.34	573.95 ±5.13	588.66 ±9.91	600.55 ±2.64	602.45 ±1.32	607.00 ±2.03

Note: M - arithmetic mean, m - standard error of mean values.

During the evaluation of live weight dynamics of steers of experimental groups of Hereford and Limousin breeds, we did not observe reliable intergroup differences in different age periods. The obtained results give a reason to exclude the influence of this polymorphism on live weight indices in different periods of ontogenesis.

The tendency of some increase in the value of the studied index in steers of both breeds with the genotype of *CAST*^{TT}. Thus, at 8 months of age, Hereford bulls of this genotype exceeded the live weight of bull calves with genotype *CAST*^{CC} – by 2.14%; at 12 months of age - by 2.05%; at 16 months of age - by 2.20%; and at 20 months of age - by 3.16%; Limousin bulls at the corresponding age - by 2.42%; 0.28%; 1.291%; and by 06%.

Thus, in postnatal ontogenesis of steers of different genotypes by SNP CAST C283T from 8 months of age there is a tendency to increase live weight in the direction of *CAST*^{CC}→*CAST*^{CT}→*CAST*^{TT}.

In the breed aspect, Limousin bull calves were characterised by a higher live weight than Hereford bulls.

The post-slaughter evaluation of carcasses obtained from Hereford and Limousin bull calves of different genotypes according to SNP CAST C283T is given in Table 4.

Table 4. Post-slaughter evaluation of carcasses obtained from bull calves of Hereford and Limousin breeds of different genotypes by *SNP CAST C283T*, (M±m)

Indicator	Breed/genotype					
	Hereford (n=15)			Limousin (n=13)		
	CC	CT	TT	CC	CT	TT
Pre-slaughter weight, kg	550.97 ±11.52	555.34 ±10.11	560.17 ±9.15	556.82 ±8.87	561.65 ±9.65	566.23 ±10.66
Steamed carcass weight, kg	320.66 ±8.11	328.21 ±9.92	334.98 ±4.11	331.86 ±6.63	336.43 ±8.23	346.60 ±12.34
Carcass yield, %	58.21	59.10	59.80	59.60	59.91	61.21
Weight of internal raw fat, kg	16.15 ±0.51	16.36 ±0.23	16.73 ±0.80	16.02 ±0.64	16.28 ±0.81	16.44 ±0.95
Fat yield, %	2.93	2.95	2.99	2.88	2.89	2.90
Slaughter weight, kg	336.81 ±7.0	344.57 ±10.6	351.71 ±9.2	347.88 ±9.5	352.71 ±11.0	363.04 ±10.0
Slaughter output, %	61.13	62.05	62.78	62.48	62.80	64.11

Note: M - arithmetic mean, m - standard error of mean values.

The results of control slaughter of experimental animals indicated that there were no significant differences between the post-slaughter evaluation of meat productivity of carcasses obtained from bulls with different genotypes of SNP CAST C283T. At the same time, pre-slaughter live weight, carcass weight and slaughter weight of carcasses obtained from bulls of Hereford and Limousin breeds with genotype $CAST^{TT}$ resulted bigger. Thus, pre-slaughter live weight of the Hereford breed exceeded the value of this indicator in steers with genotype $CAST^{CC}$ by 1.64%, and $CAST^{CT}$ – by 0.86 %, Limousin breed - by 1.66 % and 0.81 %, respectively. In terms of carcass weight, animals of Hereford breed with genotype $CAST^{TT}$ outperformed animals with the genotypes $CAST^{CC}$ and $CAST^{CT}$ - by 4.27 % and 2.02 %; Limousin breed - by 4.25 % and 2.93 %; slaughter weight - by 4.24 % and 2.03 %, 4.16 % and 2.84 %, respectively.

When studying the chemical composition of the longest muscle of the back in the meat of animals of different genotypes according to SNP CAST C283T, no significant intergroup differences in the content of dry matter and moisture were found. High dry matter content was observed in the meat of animals with genotype $CAST^{TT}$, as well as high moisture content in animals of both studied breeds with genotype $CAST^{CC}$. Protein and fat content increased in the direction: $CAST^{CC} \rightarrow CAST^{CT} \rightarrow CAST^{TT}$.

In the study of structural and mechanical properties, a significant difference was found between steers of different genotypes at the end of 1 day and on the 7th day after slaughter. When cutting the longest muscle of the back from bulls of Hereford and Limousin breeds with genotype $CAST^{TT}$ at the end of the first day required less effort than animals with genotype $CAST^{CC}$ ($p \leq 0.05$) respectively by 0.07 kg/m² (7.69 %) and 0.06 kg/m² (6.59 %); on the seventh day of the experiment - by 0.05 kg/m² (10 %) and 0.04 kg/m² (8 %). These results are in agreement with the data obtained by other researchers.

4 Discussion

The polymorphism of the calpastatin gene is actively investigated, as some of the poorly studied polymorphisms can be closely associated with certain economic and useful traits and used as DNA markers.

The results of genotyping of Hereford and Limousin bulls by SNP CAST C283T, indicate a high frequency of occurrence of the genotype in both herds $CAST^{CC}$ and low frequency of the desired genotype $CAST^{TT}$ (7.5 per cent in Herefords and 4.18 per cent in Limousins), a low frequency of the allele was naturally observed $CAST^T$ (0.29 and 0.26). The obtained results are in agreement with the results of other scientists. Thus, in the study of Gábor M. et al. [9], in Simmental cattle the frequency of allele $CAST^C$ occurrence – 0.65, allele $CAST^T$ – 0.35. The analysis of Kalmyk cattle breed on the presence of polymorphism of CAST gene showed a lower degree of occurrence of polymorphism of this gene, namely, among 50 animals 7 (16 %) animals with TT genotype (desirable), 35 (70 %) animals with ST genotype and 8 individuals with CC genotype (14 %) were detected. The frequency of the C allele was 0.51 and the frequency of the T allele was 0.49 [16]. There was a correspondence between the observed and expected distribution of genotypes, which indicates the equilibrium of the studied beef cattle herds.

No significant correlation of the studied SNP CAST C283T with weight growth traits of steers of different genotypes was found during the research. In postnatal ontogenesis of steers of different genotypes from 8 months of age there is a tendency to increase live weight in the direction: $CAST^{CC} \rightarrow CAST^{CT} \rightarrow CAST^{TT}$. A similar trend was observed in the study of post-slaughter parameters, so carcasses obtained from steers with the genotype $CAST^{TT}$, also relatively high fat content was observed in these carcasses. In the breed aspect, carcasses of Hereford cattle contained more fatty tissue. When examining the chemical composition, a high dry matter content was observed in meat from animals with

the genotype $CAST^{TT}$, protein and fat content increased in the direction: $CAST^{CC} \rightarrow CAST^{CT} \rightarrow CAST^{TT}$. The study of structural and mechanical properties revealed a significant difference between steers of different genotypes at the end of 1 day and on the 3rd day after slaughter. The longest muscle of the back of steers of both breeds with genotype $CAST^{TT}$ at the end of the first twenty-four hours required less cutting effort than the $CAST^{CC}$ ($p \leq 0.05$) respectively by 0.07 kg/m² (7.69 %) in Herefords and 0.06 kg/m² (6.59 %) in Limousins; on the third day of the experiment - by 0.05 kg/m² (10 %) and 0.04 kg/m² (8 %), respectively. The results are in agreement with the research data of other scientists [16] who have established the relationship of this polymorphism with changes in the structural and mechanical properties of cattle meat during ripening.

5 Conclusion

1. The results of genotyping of bulls of Hereford and Limousin breeds by SNP CAST C283T, indicate a high frequency of occurrence in both herds of the genotype $CAST^{CC}$ and low desirable genotype $CAST^{TT}$ (7.5% in Herefords and 4.18% in Limousins), a low frequency of the allele $CAST^T$ was noted (0.29 and 0.26).

2. In our studies, no significant correlation of the studied SNP CAST C283T with weight growth traits of steers of different genotypes was found. In the postnatal ontogenesis of steers of different genotypes from 8 months of age to the age of meat condition, there is a tendency to increase live weight in the direction $CAST^{CC} \rightarrow CAST^{CT} \rightarrow CAST^{TT}$. A similar trend was observed in the study of post-slaughter parameters, so carcasses obtained from steers with the genotype of $CAST^{TT}$, also relatively high fat content was observed in these carcasses.

3. When examining the chemical composition, a high dry matter content was observed in the meat of animals with the genotype $CAST^{TT}$, protein and fat content increased in the direction $CAST^{CC} \rightarrow CAST^{CT} \rightarrow CAST^{TT}$.

4. In the study of structural and mechanical properties of the longest muscle of the back in steers of both breeds with genotype $CAST^{TT}$ at the end of the first twenty-four hours, less effort was required in cutting than $CAST^{CC}$ ($p \leq 0.05$) respectively by 0.07 kg/m² (7.69 %) in Herefords and 0.06 kg/m² (6.59 %) in Limousins; on the third day of the experiment - by 0.05 kg/m² (10 %) and 0.04 kg/m² (8 %), respectively. This fact allows us to recommend SNP CAST C283T as a marker of beef "tenderness" in selection work with cattle of Hereford and Limousin breeds.

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