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Assessment of the occurrence of gene polymorphisms *CAPN316* and *UoGCAST* in the population of cattle

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ABSTRACT
To solve the problem of increasing the rate of breeding, but reduce the breeding work with farm animals, it is necessary to form herds with the desired level of productivity, that are adapted to specific regions of breeding, resistant to various diseases, with a decrease in the time for the breeding process. The active use of molecular genetic methods has contributed to the expansion of the list of DNA markers for farm animals, which are candidate genes for economically useful traits. Among these genes are widely known members of the calpain-calpastatin system, which is associated with postmortem proteolysis and tenderisation of muscles. The calpain system consists of an actively expressed μ-calpain (CAPN1) and m-calpain (CAPN2) and a single endogenous inhibitor, CAPN1 and CAPN2-calpastatin (CAST). The study of polymorphisms of these genes contributes to the expansion of marker characterisation in breeding. DNA samples (n=139) obtained from the blood of cattle were used in the work. Real-time PCRs were carried out using a ANK-32 programmable thermocycler (Synthol, Moscow, Russia). The CAPN316 gene polymorphism was present in 15% of animals tested, with allele G being found in 85% of animals. Similar calculations on the occurrence of the UoGCAST gene polymorphism in this sample of animals found the desirable allele G in 22% of animals and allele C in the remaining 78%. From the analysis of the occurrence of both genes and their polymorphisms in the study population, animals that had a combination of both desirable genotypes (CC*GG*) of CAPN316 and UoGCAST genes were not identified. There were also no animals that had the desired CAPN316 CC genotype and the heterozygous state of the UoGCAST gene (CC*GC). While 7.6% of the animals were CC*GG for the desirable expression of gene CAPN316, they contained the homozygous form of the gene UoGCAST.
Keywords: *genetic markers, cattle, CAPN1, CAST, DNA, tenderization.*

1. INTRODUCTION
To solve the problem of increasing the rate of breeding, but reduce the breeding work with farm animals, it is necessary to form herds with the desired level of productivity, that are adapted to specific regions of breeding, resistant to various diseases, with a decrease in the time for the breeding process. Developments in molecular biology make it possible to control the variability of quantitative polygenic traits for the calculation of breeding indices, in order to assess the breeding value of animals. The active use of molecular genetic methods has contributed to the expansion of the list of DNA markers for farm animals, which are candidate genes for economically useful traits. Identification of associations between polymorphism of complex molecular genetic markers and variability of economically valuable traits in animals allows us to anticipate that the inclusion of complex genotypes in the evaluation of the breeding indices of animals could provide a refinement in forecasting breeding value.

The genes controlling meat characteristics can be divided into those for particular traits, and those whose products can be considered as system regulators. Among these genes are widely known members of the calpain-calpastatin system, which is associated with postmortem proteolysis and tenderisation of muscles [1]. The calpain system consists of an actively expressed μ-calpain (CAPN1) and m-calpain (CAPN2) and a single endogenous inhibitor, CAPN1 and CAPN2-calpastatin (CAST) [2, 3].

Calpains, intracellular calcium-dependent cysteine proteases, are involved in muscle growth and development in mammals. In particular, the gene controlling a specific variant of calpain for skeletal muscle (CAPN3 or P94) has been studied in recent years, as this protein is involved in the formation of giant filaments essential for the structure and growth of myofibrils [4]. In 2010, Liu et al. established that the polymorphism (C36127T) of gene CAST was related to qualitative indicators of meat and CAPN1. It was shown that the expression of these genes had an effect on muscle regeneration [5]. Therefore, the study of polymorphisms of these genes contributes to the expansion of marker characterisation in breeding.

2. MATERIALS AND METHODS
2.1. Samples.
Five ml whole blood samples were taken from animals (n = 139) using a disposable instrument and 1.5 M EDTA as an anticoagulant Genomic DNA was isolated using a DNA-Extran 1 kit (Syntol, Moscow, Russia), according to the manufacturer's instructions. The quality and quantity of nucleic acid was measured using a Nanodrop® ND-1000 spectrophotometer (Thermo Fisher Scientific, Waltham, USA). Genomic DNA of each animal was stored at -20 °C.

2.2. Polymerase chain reaction (PCR) amplification.

Primers were designed based on published sequences for *CAPN1* (GenBank Accession Nos. AF252504) and *CAST* (GenBank Accession Nos. AY008267), using Primer3 software (www.genome.wi.mit.edu) Table I.

Real-time PCRs were carried out using a ANK-32 programmable thermocycler (Synthol, Moscow, Russia) in a total reaction mixture of 25 µl, containing 60 mM Tris-HCl (pH 8.5), 1.5 mM $MgCl_2$, 25 mM KCl, 10 mM mercaptoethanol, 0.1 mM Triton X-100, 0.2 mM dNTPs, 1 unit Taq DNA polymerase and 0.5 µM of each primer. The single nucleotide polymorphism (SNP) amplification of *CAPN1* and *CAST* genes was performed according to the protocols specified in Table 2.

2.3. Statistical processing.

The frequency of genotypes was determined by equation (1):

$$p = n/N \qquad (1)$$

where p is the frequency of genotype determination, n is the number of individuals with a specific genotype, and N is the number of individuals.

The frequency of individual alleles was determined by equations (2) and (3):

$$PA = (2nAA + nAB) / 2N \qquad (2)$$

$$qB = (2nBB + nAB) / 2N \qquad (3)$$

where PA is the frequency of allele A, qB is the frequency of allele B, and N is the total number of alleles.

The expected results of genotype frequencies in the studied population were calculated according to the Hardy-Weinberg principle. The results were processed by the biometric method using standard programs.

Statistical processing was carried out using Statistica 10.0 software (StatSoft, Tulsa, OK, USA). The analysis included the determination of the arithmetic mean (M) and the standard error of the mean (SE). Differences were considered significant when $P = 0.05$. The statistical significance of the differences between the groups was assessed using Student's t-test.

Table 1. SNP name and location

Gene	SNP name	Location	GenBank (Accession number and base position)	SNP
[1]CAPN1	*CAPN316*	Exon 9	AF252504-5709	G/C
[2]*CAST*	*UoGCAST*	Intron 5 between exon 5 and 6	AY008267-282	G/C

[1]Calpain was mapped by Smith *et al*., (2000) [6.], *CAPN316* SNP reported by Page *et al.*, (2002) [7], [2] Calpastatin was mapped by Bishop *et al.*, (1993) [8] and the SNP was reported by Schenkel *et al*., (2006) [9]

Table 2. Programs of DNA amplification

SNP name	Temperature, ºC	Cycle
CAPN316	95 ºC	120 s x 1
	63ºC	40 s x 40
	95ºC	20 s x 40
UoGCAST	95 ºC	120 s x 1
	63ºC	40 s x 40
	95ºC	15 s x 40

3. RESULTS

The CAPN316 gene polymorphism was present in 15% of animals tested, with allele G being found in 85% of animals. Similar calculations on the occurrence of the UoGCAST gene polymorphism in this sample of animals found the desirable allele G in 22% of animals and allele C in the remaining 78% (Table 3). Therefore, the frequency of the desired allele of the UoGCAST gene polymorphism was higher than that of the CAPN316 gene polymorphism.

Since such data on alleles was not sufficient to assess the prevalence of gene polymorphisms CAPN316 and UoGCAST, further analysis was conducted on the occurrence of the desirable genotypes in the studied micro-population. This revealed that the incidence of the desired genotype of the CC gene CAPN316 was 7%, while the remaining genotypes consisted of 73% in the homozygous state GG and 20% as the heterozygote (GC). The frequency of occurrence of the polymorphism CC genotype UoGCAST was 62%, and that of the heterozygous manifestation was 23%, while the desirable genotype GG accounted for the remaining 15%. Thus, it could be concluded that the desired genotype in the gene polymorphism UoGCAST was more common than that for gene CAPN316. At the same time, homozygous and heterozygous manifestation of these genes differed slightly.

From the analysis of the occurrence of both genes and their polymorphisms in the study population, animals that had a combination of both desirable genotypes (CC*GG*) of CAPN316 and UoGCAST genes were not identified. There were also no animals that had the desired CAPN316 CC genotype and the heterozygous state of the UoGCAST gene (CC*GC). While 7.6% of the animals were CC*GG for the desirable expression of gene CAPN316, they contained the homozygous form of the gene UoGCAST.

Only 2.5% of animals had the desirable genotype of GG* for the UoGCAST gene with the heterozygous state of the CAPN316 gene (CGGG*). Those with a combination of homozygous genotypes (GGGG*) and a combination of heterozygous genotypes in both genes (GCGC) represented 13% and 7.6% of the general population, respectively.

More animals from the study population (46%) had homozygous manifestation of wild-type genotypes of both genes (GGCC). The manifestation of the combination of genotypes GCCC and GGGC was 10.2 and 12.8%, respectively.

In summary, we concluded that the majority of animals have a wild-type manifestation of both genes; however, other combinations of genotypes could add to the breeding portfolio.

Analysis of candidate genes responsible for the formation of high-quality meat traits is one of the priorities of modern science. Many studies have demonstrated the relationship between *CAPN1* and *CAST* genes.

Notably, Voges et al. have shown that the calpain-calpastatin system can improve the sensitivity of muscle after slaughter by splitting the limiting myofibrillar proteins, such as titin, desmin and vinculin, while high levels of CAST are related to a decrease in proteolysis and an increase in the stiffness of meat [10]. Thus, CAPN1 and CAST probably have a significant impact on the tenderness of meat. In fact, the genotypic effects of *CAPN1* could significantly affect carcasses of many species. Numerous other studies have shown that the calpain system plays an important role in the quality of carcasses and tenderness of meat [11, 12].

Table 3. Genotypic and allelic frequencies for SNP

SNP	Genotype	Genotype frequency	Allele	Allele frequency
CAPN316	GG	73±0,3	G	85±0,3
	GC	20±0,24	C	15±0,24
	CC*	7±0,3	-	-
UoGCAST	GG*	15±0,1	G	22±0,1
	GC	23±0,18	C	78±0,18
	CC	62±0,1	-	-

* - desirable genotype

4. CONCLUSIONS

Thus, the obtained results allow to state the fact of low frequency of occurrence of desirable genotypes of economically useful signs in the population of cattle. Accordingly, it can be assumed that an increase in the actual number of heterozygotes contributes to a greater probability of obtaining the desired genotype of animals. Thus, assessing the presence and the degree of prevalence of candidate genes economically useful features can be carried out qualitatively selection process.

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6. ACKNOWLEDGEMENTS

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