AGRICULTURAL BIOLOGY,
2023, V. 58, Iss. 4, pp. 622-637
[SEL'SKOKHOZYAISTVENNAYA BIOLOGIYA]

ISSN 2412-0324 (English ed. Online)
ISSN 0131-6397 (Russian ed. Print)
ISSN 2313-4836 (Russian ed. Online)

Population genetic structure

UDC 636.2.033:575.174

doi: 10.15389/agrobiology.2023.4.622eng
doi: 10.15389/agrobiology.2023.4.622rus

DNA ANALYSIS OF MYOSTATIN, LEPTIN AND CALPAIN 1 GENE POLYMORPHISM IN RUSSIAN CATTLE POPULATION OF ABERDEEN ANGUS BREED

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The authors declare no conflict of interests
Acknowledgements:
The research was carried out within the framework of the state assignment of the Ministry of Science and Higher Education of the Russian Federation No. 121052600344-8.
Final revision received December 20, 2022
Accepted January 30, 2023

Abstract

Beef meat is characterized by high nutritional value and unique amino acid composition (V.S. Kolodyaznaya et al., 2011; D. Pighin et al., 2016). Specialized beef cattle breeds in particular Aberdeen Angus ensures good acclimatization ability and high productivity both in Russia and broad (R. Toušová et al., 2015; V.M. Gabidulin et al., 2018; A.I. Otarov et al., 2021). Modern strategies of increasing efficiency of meat cattle farming include animal genotyping for genetic determinants of high productivity and targeted selection (V.F. Fedorenko et al., 2018; S.A. Terry et al., 2020). This paper is the first to report the development of a PCR-RFLP-based test for detection of Arg4Cys *LEP* and *CAPN1*_316 allele polymorphisms. This test was used for genotyping of the Aberdeen-Angus cattle population. The frequencies of the different polymorphism genotypes are evaluated and the influence of the *LEP* and *CAPN1* polymorphisms on animal fattening qualities. The purpose of the study was to develop the test systems for revealing the leptin *LEP* gene c.466 C→T polymorphism and calpain 1 *CAPN1* gene polymorphism rs17872000, to genotype Aberdeen Angus cattle population for these genes and the myostatin *MSTN* gene, and to investigate the links between different allele variants and fattening qualities of animals. Bulls (n = 64) and heifers (n = 81) of the population of Aberdeen-Angus young cattle (*Bos taurus*) (OOO Happy Farm, Medynsky District, Kaluga Province) born from March 2020 until May 2021 were selected for the study. Weaning of calves from mothers was carried out at the age of 6-9 months, depending on the development of a particular calf. The live weight was determined at the age of 6, 8, 12 and 15 months. Blood samples were collected for genotyping. DNA was isolated using DNA-Extran 1 kit (ZAO Syntol, Russia). The test for genotyping was developed based on the polymerase chain reaction—restriction fragment length polymorphism (PCR-PDRF) method. PCR amplification was carried out on a Bio-Rad T100 thermal cycler (Bio-Rad Laboratories Singapore) followed by RFLP analysis of amplification products to differentiate the SNP alleles. Specific endonucleases with restriction sites in mutant alleles were found using the NEBcutter v2.0 program (https://nc2.neb.com/NEBcutter2/). PCR-RFLP products were analyzed using 2 % agarose gel electrophoresis. The test system for the F94L *MSTN* polymorphism has been developed earlier (E.N. Konovalova et al., 2021). To verify the *CAPN1*_316 amplification, Sanger sequencing was performed for three genotypes. In developing the diagnostic test for of Arg4Cys *LEP* и *CAPN1*_316 polymorphisms, we used the sequences of NM_174259 and AJ512638.1 (NCBI, https://www.ncbi.nlm.nih.gov/). The polymorphism F94L *MSTN CC* genotype occurred in 98.77 % of investigated animals. For Arg4Cys *LEP* polymorphism, *CT* genotype prevailed with a frequency of 42.19 % for bulls and 45.68 % for heifers. For *CAPN1*_316 polymorphism, the *GG* genotype predominated and accounted for 51.56 % of bulls and 69.14 % of heifers. The frequencies of the desirable productivity alleles among bulls and heifers were 0.00 and 0.01, respectively, for *A* F94L *MSTN*, 0.49 and 0.51 for *C* Arg4Cys *LEP*, and 0.20 and

0.28 for *C CAPN1*_316. The bulls with Arg4Cys *LEP TT* genotype demonstrated the highest efficiency of live weight gain from birth to 8 months, the 778 g per day which is significantly higher ($t = 2.18$) compared to *CC* (748 g). For *CAPN1*_316 polymorphism, in heifers from birth to 8 months, there was a trend to higher daily weight gain in animals of *CC* genotype, 770 g vs. 720 g for *GC* genotype and 730 g for *GG* genotypes. However, in the post-weaning period, the observed trends changed. The 12-month-old bulls with the Arg4Cys *LEP CC* genotype had a significantly higher live weight compared to *CT* bulls, The *CAPN1*_316 *GG* heifers of 8 to 15 months of aged showed a significantly higher live weight gain compared to *CAPN1*_316 *CC*, 790 g vs. 740 g. The developed test systems ensures detection of the Arg4Cys *LEP* and *CAPN1*_316 polymorphisms and can be used for genotyping and selecting beef cattle of desirable genotypes.

Keywords: cattle, Aberdeen Angus breed, productivity markers, SNP, myostatin, leptin, calpain 1

Beef is characterized by a high protein content, balanced in six essential amino acids, and the presence of two limiting amino acids, the leucine and threonine, which makes it most suitable for dietary nutrition [1]. Beef is an important source of linoleic acid, which has anticarcinogenic activity, and contains transisomers of fatty acids that help prevent coronary heart disease [2].

Currently, there are approx. 1 billion cattle in the world for meat production. Compared to poultry and swine, beef cattle have a significantly lower efficiency in converting feed into muscle tissue. However, it should be taken into account that beef cattle produce high-quality protein mainly from those feeds that are either not used or make up a small proportion in the diet of pigs and poultry [3].

In addition to feeding, housing and breeding, modern strategies for improving the efficiency of beef production focus on the genetic traits of animals that have a significant impact on their health and productivity [3]. Nowadays. genotyping techniques developed due to modern advances in molecular biology are used to establish the genetic determinants of high animal productivity for targeted selection of carriers of economically valuable alleles to involve them in breeding [4].

Specialized beef cattle breeding in Russia has undergone dynamic development in the last decade due to special program and significant government support [5]. Because of high nutritional value, increasing the proportion of beef from specialized beef cattle breeds is important for improving the quality of food supply for the population [3].

Aberdeen Angus are a highly productive beef cattle breed that have become widespread throughout the world [5]. Animals of this breed are distinguished by high growth energy. The average daily increase in live weight is more than 1000 g, and in the best individuals, it can reach 1300-1500 g [6]. In addition, characteristic features of the Aberdeen Angus breed, which are transmitted through crossing with other breeds, include polling, tender, fine-fiber meat with well-defined marbling [7, 8].

In the Kaluga Province, as in many regions of the Russian Federation, beef cattle is one of the priority for the development of livestock breeding. Necessary infrastructure, favorable natural and climatic conditions, a stable sales market, and grant supports promote a significant increase in the number of specialized beef cattle, from 24,411 animals in 2016 to 104,459 animals by 2020, or by 427.9% [9]. The dynamic development of the industry in this region, in particular at Peasant Happy Farm LLC (Kaluga Province, Medynsky District) which currently specializes in breeding Aberdeen Angus cattle, requires the modern genetic methods for breeding, including genotyping for genes associated with productive traits. These genes in beef cattle include myostatin (*MSTN*), leptin (*LEP*) and calpain 1 (*CAPN1*).

Myostatin plays a key role in skeletal muscle development. After animal birth, the myostatin gene negatively regulates skeletal muscle growth and development by limiting the number and size of muscle fibers [10]. Myostatin and its

effect on meat yield have been intensively studied, primarily with regard to loss-of-function mutations leading to increased skeletal muscle mass and appearance of the double-muscle phenotype which has been described in many species, including cattle, sheep, pigs, rabbits, and humans [11].

In cattle, the *MSTN* gene, also known as differentiation factor 8 (*GDF8*), is located on chromosome 2 (BTA2), and of the nine *MSTN* mutations currently known in *Bos taurus* [12], two have been found in the Aberdeen Angus breed. These are deletion 11 bp (nt821del11) which causes the genetic defect of double muscularity (M1) and single nucleotide polymorphism F94L (c.282C>A), causing the amino acid replacement of phenylalanine with leucine. Both mutations have a similar effect on intensive muscle growth, however, F94L, unlike nt821del11, is not associated with increased bodyweight of the calf at birth, which allowed foreign experts to recommend to include it in breeding programs to improve the accuracy of genomic prediction [13-15]. Previously, a fairly high frequency of occurrence of the desired allele *A* (0.97-1.00) in Russian populations of Limousin cattle was shown [16]. This polymorphism can be considered a promising genetic marker for selecting animals with increased muscle mass.

Quite interesting is the leptin gene (*LEP*) which is also called the obesity gene. The *LEP* gene produces the hormone of the same name which is secreted by adipocytes. Its connection with the feed intake and energy balance in mice and humans has been established [17]. In cattle, this gene is located on chromosome 4 (BTA4). The Arg4Cys *LEP* c.466 C→T polymorphism, also referred to as LEP73, R4C and R25C, is of immediate interest. It is located at a 73 bp distance from the beginning of exon 2 and causes the amino acid substitution of arginine for cysteine at position 4 of mature leptin [18-20]. The *T* allele of this SNP is associated with increased leptin mRNA expression which directly affects feeding behavior, determines the greatest efficiency of feed consumption and, as a consequence, formation of carcasses with a high fat content [21]. The *C* allele is associated with less fat deposition in the carcass, resulting in leaner carcasses produced from animals carrying this genotype [22].

Protein hydrolysis is known to be closely related to muscle growth during fattening and meat tenderness after slaughter. Calpain 1 (CAPN1) is an important protease that hydrolyzes proteins in myofibers [23]. In this regard, one of the polymorphisms of the calpain 1 gene, a single nucleotide substitution c.947G>C *CAPN1*_316 located on BTA29 is being considered. Due to this SNP, a variant of the calpain 1 protein is encoded that causes the weakening of connections between muscle fibers. This provides the uniform distribution of intramuscular fat and, thereof, greater marbling, tenderness and juiciness of meat [24-27].

The study of genes associated with meat productivity and the development of test systems for identifying preferential allelic variants remain relevant. It is necessary to take into account that the availability and ease of genotyping method is important for mass use in breeding beef cattle.

In this work, test systems were created based on the PCR-RFLP method (polymerase chain reaction—restriction fragment length polymorphism) to identify allelic variants of the Arg4Cys *LEP* and *CAPN1*_316 polymorphisms. The developed tests were used for genotyping of the Aberdeen Angus cattle population. The frequencies of occurrence of various genotypes on the studied polymorphisms were calculated, and the influence of polymorphisms in the leptin and calpain 1 genes on the fattening qualities of animals was assessed.

Our goal was i) to develop test systems for detecting polymorphisms of the leptin (*LEP*) gene c.466 C→T and calpain 1 (*CAPN1*) gene rs17872000, ii) to investigate the Aberdeen Angus cattle population for these genes and the myostatin gene (*MSTN*), and iii) to reveal the relationship of allelic variants with

the fattening qualities of animals.

Materials and methods. The work was carried within the framework of scientific cooperation of Ernst Federal Research Center for Animal Husbandry — VIZh with Timiryazev Moscow Agricultural Academy. In 2021-2022, a purebred population of young Aberdeen-Angus cattle (*Bos taurus*) of the Happy Farm LLC (Kaluga Province, Medynsky District) (n = 145) was investigated. The representative groups were bulls (n = 64) and heifers (n = 81) born between March 2020 and May 2021.

Animals were kept loose in groups, in open walking areas with light windproof canopies and feeding tables. A feed mixer was used to distribute a complete feed twice a day, providing animals with the necessary nutrients in accordance with the standards for each full-age group. Automatic heated drinking bowls were installed at the sites. Calves were weaned from their mothers at 6-9 months of age depending on theier growthe rate.

The young animals were weighed on electronic scales with fixation REUS-A-U (Tenzosila LLC, Russia) at the age of 6, 8, 12 and 15 months.

DNA for genotyping was extracted from blood sampled on September 12, 2021 and November 21, 2021 using a Vacuette blood collection system (K2E Sep tubes with EDTA and separating gel) (Greiner Bio-One, Austria). DNA was isolated using the DNA-Extran 1 kit (ZAO Synthol, Russia) in accordance with the manufacturer's protocol. Since the polymorphism of the genes under study is caused by simple single nucleotide polymorphisms (SNPs), the polymerase chain reaction method followed by restriction fragment length polymorphism analysis (PCR-RFLP) was used to develop test systems.

To amplify the mutation regions, oligonucleotide primers were selected using the Primer3Plus software (https://www.primer3plus.com/):

Polymorphism	Primer sequence (5′→3′)	Restriction endonuclease (site)
Arg4Cys *LEP*	LPN_F: TGATAGCCATGGCAGACAGC LPN_R: CCTCCCTACCGTGTGTGAGA	HpyCH4V (TG↓CA)
*CAPN1*_316	CAPN316_F: TGAACTACCAGGGCCAGATG CAPN316_R: ACAGGGTGGTGTTCCAGTTG	BstDSI (C↓CRYGG)

PCR was carried out in the following mode: 3 min at 95 °C (initial denaturation); 30 s at 95 °C (denaturation), 40 s at 61 °C (annealing) and 30 s at 72 °C (elongation) (35 cycles); 4 min at 72 °C (final elongation) (a Bio-Rad T100 thermal cycler, Bio-Rad Laboratories, Singapore). If amplification was successful, the alleles of the studied SNPs were detected by RFLP analysis using restriction endonucleases that recohnize specific sites in the DNA sequences of mutant alleles. Restriction endonucleases were chosen in the NEBcutter v2.0 program (https://nc2.neb.com/NEBcutter2/). An enzyme (1 u.a.) was added to the sample and incubated for 8 h. The enzymes HpyCH4V and BstDSI (SibEnzyme LLC, Russia) were used in accordance with the manufacturer's recommendations.

PCR-RFLP products were analyzed by electrophoresis in a 2% agarose gel for 30 min at 125 V using a molecular weight marker with 100 bp increments (ZAO Syntol, Russia).

A test system for analyzing the F94L *MSTN* polymorphism was developed previously [16].

To verify correctness of PCR for the polymorphism of the calpain 1 gene *CAPN1*_316, the amplification products of three putative genotypes were sequenced by Sanger method with the equipment and protocol of JSC Evrogen, Russia. The resulting sequences were processed using UGENE Pro V.38.1 9 software (http://ugene.net/ru/).

Genotype frequencies and average daily bodyweight gains were calculated by common methods [28, 29].

Statistical analysis was carried out according to standard methods using

the Microsoft Excel 2013 software package [30, 31]. The statistical significance of differences was assessed by Student's *t*-test. The difference was considered statistically significant at $t \geq 1.96$ and $p < 0.05$.

Results. As a result of primer design, optimized temperature-time regime of PCR and choice of restriction enzymes, test systems for the analysis of polymorphisms of the leptin (Arg4Cys *LEP*) and calpain 1 (*CAPN1*_316) genes were developed (Fig. 1, 2).

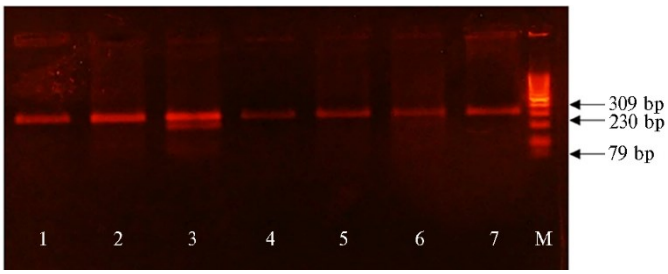

Fig. 1. Genotyping of young cattle (*Bos taurus*) of the Aberdeen Angus breed based on the polymorphism of the leptin gene Arg4Cys *LEP*. The top fragment is 309 bp long and corresponds to the wild type allele *C*, lower fragment 230 and 79 bp correspond to mutant allele *T*; 1, 2, 4-7 — animals with the *CC* genotype, 3 — with the *CT* genotype. M — molecular mass marker in 100-bp increments (ZAO Syntol, Russia) (Peasant Happy Farm, Kaluga Province, 2020-2021).

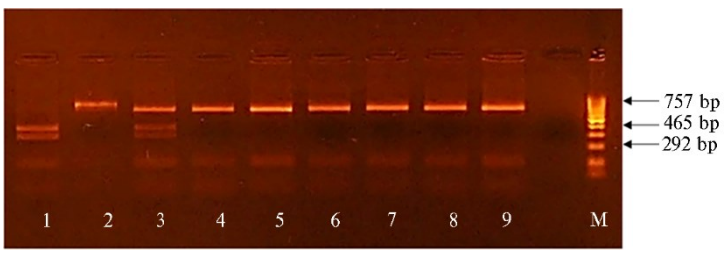

Fig. 2. Genotyping of young cattle (*Bos taurus*) of the Aberdeen Angus breed based on the polymorphism of the calpain 1 gene *CAPN1*_316. The top fragment is 757 bp long and corresponds to the wild type allele *G*, lower fragments 465 and 292 bp correspond to mutant allele *C*; 1 — animal with the *CC* genotype, 3 — with the GC genotype, 2, 4-9 — with the *GG* genotype. M — molecular mass marker in 100-bp increments. (ZAO Syntol, Russia) (Peasant Happy Farm, Kaluga Province, 2020-2021**).**

When developing a test system to detect the *CAPN1*_316 polymorphism, we used the sequence NM_174259.2 (National Center for Biotechnology Information, NCBI, https://www.ncbi.nlm.nih.gov/gene/). It was expected that for the mutant allele a DNA fragment of 267 bp would be amplified, which upon restriction would produce fragments of 164 and 103 bp. However, the resulting DNA fragments turned out to be much larger, which prompted us to conduct a comparative BLAST analysis of the NM_174259.2 sequence with the whole genome assembly (NCBI >281661-UMD3.1.1) (https://blast.ncbi.nlm.nih.gov/Blast.cgi). The analysis showed 100% identity of the compared sequences, and the sizes of the obtained amplicons corresponded to the sequence >281661-UMD3.1.1. That is, when analyzing *CAPN1*_316, a DNA fragment of 757 bp in size was amplified, which, in the presence of a mutant allele, was cut into two fragments of 465 and 292 bp in size (see Fig. 2).

Sanger sequencing of the amplified fragment of the *CAPN1*_316 mutation region revealed the desired DNA sequence and three possible genotypes for this polymorphism (Fig. 3), finally eliminating false results.

When developing a test system for diagnosing Arg4Cys *LEP* polymorphism, the NCBI AJ512638.1 sequence was used. As expected, PCR amplified a DNA fragment of 309 bp in size, which, in the presence of a mutation, was cut by the corresponding restriction endonuclease into two fragments of 230 and

79 bp in length. Thus, the developed test system ensures can clearly identify the *TT*, *CT* and *CC* genotypes of Arg4Cys *LEP*.

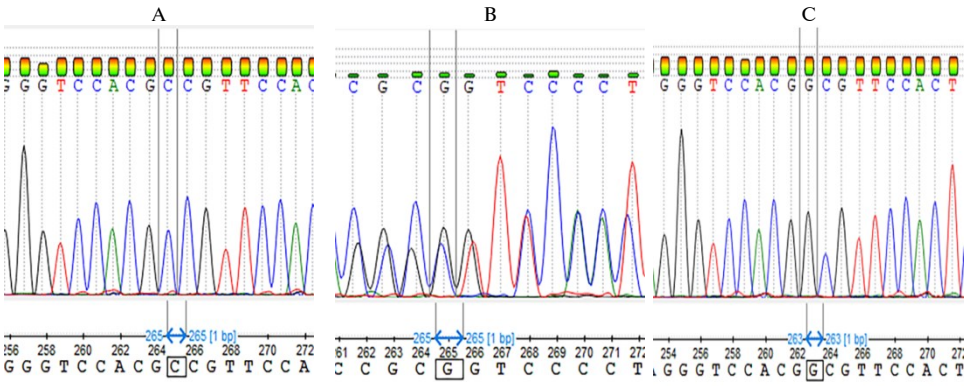

Fig. 3. Results of Sanger sequencing of the PCR fragment of the calpain 1 gene *CAPN1*_316 mutation region in young cattle (*Bos taurus*) of the Aberdeen Angus breed: A — *CC* genotype, B — *GC* genotype, C — *GG* genotype (Peasant Happy Farm, Kaluga Province, 2020-2021).

When genotyping young Aberdeen Angus breeds, we found that all the studied genes were polymorphic, but the frequency of homozygous and heterozygous genotypes among bulls and heifers varied widely (Table 1).

1. Results of Aberdeen Angus young cattle (*Bos taurus*) genotyping for polymorphisms F94L *MSTN*, Arg4Cys *LEP* and *CAPN1*_316 (Peasant Happy Farm, Kaluga Province, 2021-2022)

Polymorphism	Genotype/allele	Frequency of occurrence	
		bulls (*n*/%)	heifers (*n*/%)
F94L *MSTN*	*CC*	64/100	80/98.77
	CA	0/0.0	1/1.23
	*AA**	0/0.0	0/0.0
	C	1.00	0.99
	*A**	0.00	0.01
Arg4Cys *LEP*	*TT*	23/35.93	23/28.39
	CT	27/42.19	37/45.68
	CC	14/21.88	21/25.93
	T	0.51	0.49
	C	0.49	0.51
*CAPN1*_316	*GG*	33/51.56	56/69.14
	GC	26/40.63	18/22.22
	*CC**	5/7.81	7/8.64
	G	0.80	0.72
	*C**	0.20	0.28

N o t e. The allele and genotype desired for selection are marked with an asterisk.

Thus, based on the F94L MSTN polymorphism, two of three putative genotypes were identified, i.e., the homozygous for the wild-type allele (*CC*) and heterozygous (*CA*) which was found in only one heifer (1.23%). The *AA* genotype was not found among the studied animals.

For Arg4Cys *LEP*, all three expected genotypes were identified, while *CC* and *CT* in the groups of bulls and heifers were detected with a similar frequency (21.88 and 25.93%, 42.19 and 45.68%, respectively; 35.93% of bulls and 28.39% of heifers had the *TT* genotype.

For *CAPN1*_316, all three expected genotypes were also found, but the frequency of occurrence of the *CC* genotype, desirable for breeding, was relatively low for bulls and heifers, 7.81 and 8.64%, respectively. Most of the animals were homozygous for the *G* allele. In total, 40.63% of bulls and only 22.22% of heifers (that is, 2 times less) were heterozygous.

The frequency of the desired allele *A* for F94L *MSTN* among bulls and heifers was 0.00 and 0.01, respectively, of *C* for Arg4Cys *LEP* 0.49 and 0.51, of *C*

for *CAPN1*_316 0.20 and 0.28 (see Table. 1).

Analysis of the bodyweight dynamics allowed us to conclude that the animals under study were highly productive, while the bodyweight of bull calves at 8 months met the minimum breed requirements for elite class animals, and heifers exceeded the requirements of class I by 8.7% [32]. Thus, the average bodyweight at birth was 23.73±0.51 kg for bulls and 22.18±0.38 kg for heifers. At the age of 6 months it reached 181.18±1.98 kg in bulls and 168.38±1.47 kg in heifers, at 8 months 220.69±2.85 and 201.14±1.89 kg, at 12 months 334.11±4.25 and 317.00±13.4 kg, and at 15 months 409.38±4.81 and 368.89±4.48 kg. The average daily weight gain was over the period from birth to 8 months 803 g for bulls and 733 g for heifers, from 8 to 15 months 886 and 784 g. The analysis of the productivity of young animals indicated that favorable conditions were created on the farm to realize the productivity potential and the possibility of obtaining representative data when comparing indicators in animals with different genotypes for the *MSTN*, *LEP* and *CAPN1* genes.

2. Dynamics of bodyweight (kg) **of the Aberdeen-Angus young cattle (*Bos taurus*) depending on genotypes for the Arg4Cys *LEP* and *CAPN1*_316 polymorphisms** (*M*±SEM, Peasant Happy Farm, Kaluga Province, 2021-2022)

SNP	Genotype	Age, months				
		0	6	8	12	15
		Bulls				
Arg4Cys *LEP*	*TT* (*n* = 23)	22.74±0.70	185.36±3.26	225.79±4.77	337.52±3.70	414.83±4.88
	CT (*n* = 27)	24.59±0.86	179.13±2.85	214.45±3.80	327.33±2.36	403.37±3.72
	CC (*n* = 14)	23.71±1.31	179.95±4.86	219.79±7.20	341.57±6.19	417.57±7.55
t	*CC* и *CT*	0.62	0.15	0.66	2.13*	1.69
	CT и *TT*	1.67	1.21	1.86	2.27*	1.87
	CC и *TT*	0.73	0.75	0.69	0.56	0.31
*CAPN1*_316	*GG* (*n* = 33)	23.79±0.71	179.66±2.64	218.06±3.98	334.79±2.63	412.18±3.58
	GC (*n* = 26)	23.73±0.79	184.75±3.27	224.04±4.57	337.19±3.61	413.35±4.56
	CC (*n* = 5)	23.40±2.72	172.73±5.69	207.86±7.63	313.60±11.29	358.80±14.46
t	*CC* и *CG*	0.14	1.83	1.82	1.99*	1.82
	CG и *GG*	0.05	1.21	0.99	0.54	0.20
	CC и *GG*	0.16	1.10	1.18	1.83	1.77
		Heifers				
Arg4Cys *LEP*	*TT* (*n* = 23)	21.16±0.51	171,18±2,62	205,42±3,26	313,90±3,44	368,84±5,27
	CT (*n* = 37)	22.13±0.53	166,56±2,21	199,01±2,73	316,28±2,53	370,28±2,53
	CC (*n* = 21)	23.48±0.90	167,00±3,62	199,08±4,86	320,57±2,83	367,81±4,51
t	*CC* и *CT*	1.29	0,10	0,01	1,13	0,48
	CT и *TT*	1.31	1,35	1,51	0,56	0,25
	CC и *TT*	2.23	0,94	1,08	1,50	0,15
*CAPN1*_316	*GG* (*n* = 56)	22.39±0.48	168,03±1,94	200,92±2,49	318,37±1,87	369,80±2,94
	GC (*n* = 18)	22.88±0.80	164,64±3,54	197,85±4,58	312,65±4,43	368,53±5,18
	CC (*n* = 7)	19.50±0.50	176,14±2,07	207,24±5,55	317,00±2,77	366,00±6,42
t	*CC* и *CG*	3.60*	2,80*	1,30	0,83	0,31
	CG и *GG*	0.53	0,84	0,59	1,19	0,21
	CC и *GG*	4.18*	2,86*	1,04	0,41	0,54

* The differences are statistically significant at $t \geq 1.96$ and $p < 0.05$.

The study of the dynamics of live weight should be divided into two periods, before weaning from birth to 8 months and after weaning dueing 12-15 months, associated with the technology of raising calves (Table 2).

According to the Arg4Cys *LEP* polymorphism in the group of bulls up to 8 months, the greatest tendency to increase bodyweight was in animals with the *TT* genotype, which at the same time had the lowest birth weight. Thus, bulls with the *TT* genotype were heavier than their counterparts with the *CC* genotype by 5.41 kg at 6 months of age, and by 6.00 kg at 8 months of age. A similar trend was revealed in heifers with the *TT* genotype, at the age of 6 months they weighed 4.18 kg more than their *CC* counterparts, and at 8 months 6.34 kg more. At birth, heifers with the *TT* genotype weighed 2.32 kg less compared to the *CC* genotype ($t = 2.23$).

In the post-weaning period (see Table 2), the observed trend changed: at

the age of 12 months, bulls with the *TT* genotype were inferior in bodyweight to those homozygous for the *C* allele by 4.05 kg, but were heavier than their analogues with the *CT* genotype by 10.19 kg ($t = 2.27$). At 15 months, the difference between the *TT* and *CC* genotypes was 2.74 kg in favor of homozygotes for the *C* allele. The minimum bodyweight was in heterozygous animals. In the heifers at 12 months, there was also an advantage of animals with the *CC* genotype over *TT* genotype by 6.67 kg. At 15 months, heifers with the *TT* genotype again weighed 1.03 kg more than their *CC* counterparts.

It is noteworthy that animals heterozygous for the *LEP* gene polymorphism had the lowest bodyweight values from birth to 15 months in both sex and age groups.

For the *CAPN1*_316 polymorphism in the bulls, the highest live weight was in heterozygous animals, at the age of 6 and 15 months it was 172.73±5.69 and 358.80±14.46 kg, respectively (see Table 2). However, this superiority was not statistically significant and only indicated a trend. The animals of the *CC* genotype showed the lowest rates, their bodyweight at 12 months was 21.18 kg lower compared to the *GG* genotype *(t* = 1.99). In the heifers, a statistically significant advantage was observed for animals with the *C* allele of *CAPN1*_316 in terms of bodyweight at birth and at the age of 6 months. Thus, in animals with the ***CC*** genotype, despite a significantly less weight at birth (by 2.89 kg vs. *GC*, $t = 3.60$ and by 3.38 kg vs. *GG*, $t = 4.18$), at 6 months of age haf more weight (by 8.11 kg vs. *GG*, $t = 2.86$ and by 11.5 kg vs. *GC*, $t = 2.80$).

In the Arg4Cys *LEP* locus, the *T* allele in the homozygous state had the most pronounced effect on the average daily weight gain from birth to 8 months of age. Among bulls, individuals with the *TT* genotype gained 778 g daily, which was 50 g more compared to *ST* bulls ($t = 2.18$) and 30 g more compared to *CC* bulls. In heifers under 8 months of age, a similar trend occurred, the animals with the *TT* genotype gained 755 g vs. 725 g for the *ST* genotype and 720 for the *CC* genotype.

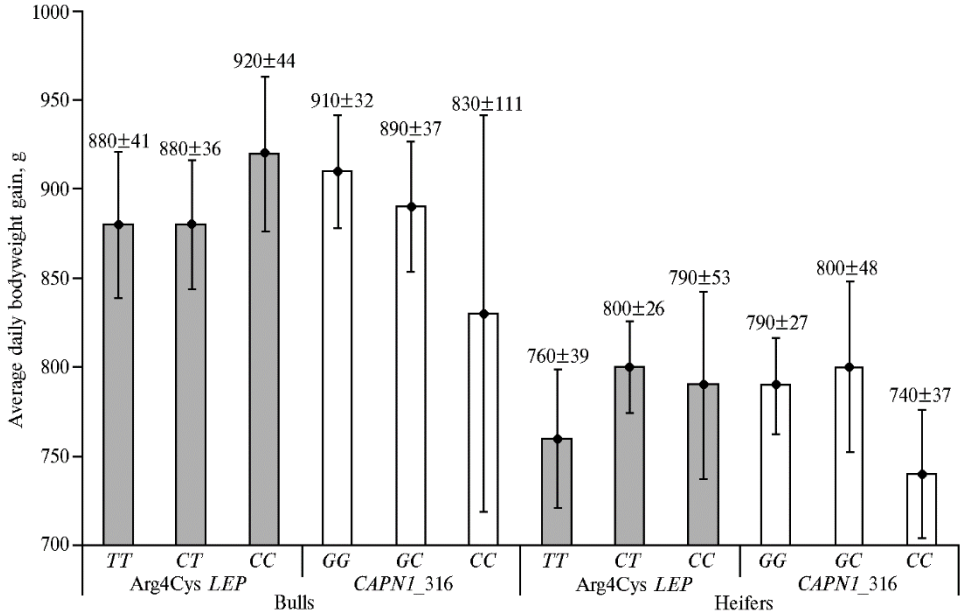

Fig. 4. Average daily bodyweight gain in the Aberdeen-Angus young cattle (*Bos taurus*) depending on genotypes for polymorphisms of the leptin Arg4Cys *LEP* and calpain 1 *CAPN1*_316 genes (*M*±SEM, Peasant Happy Farm, 2021-2022). See Table 2 for sample sizes.

At the *CAPN1*_316 locus, a positive effect of the *C* allele in the homozygous

state was found among heifers. In average daily weight gain, the *CC* cariers under the age of 8 months were superior to *GG* and *GC* by 40 and 50 g, respectively.

However, in the post-weaning period (8-15 months), the observed trends changed. For Arg4Cys *LEP*, there was an advantage in average daily weight gain in the *CC* bulls. They gained 40 g/day more compared to the *CT* and *TT* genotypes. Among heifers, the largest average daily weight gain occurred in heterozygous animals. For *CAPN1*_316, the maximum values among heifers were recorded for the *GC* genotype, and among bulls for the *GG* genotype (Fig. 4).

The developed test systems, based on a technically relatively simple and least expensive method of PCR-RFLP analysis, is the most accessible means of animal genetic identification for use in any Russian or foreign molecular genetic laboratory without any restrictions. It should be noted that the number of genotype identification systems based on fluorescent detection is growing every year. PCR-RFLP method was used to detect the Arg4Cys *LEP* polymorphism [23]. To ,detect the *CAPN1*_316 polymorphism, along with PCR-RFLP [27], real-time PCR or matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) methods were used [24-26]. The main advantages of fluorescent methods are the avoiding of agarose gel electrophoresis step and automation, which reduces analysis time for high-throughput genotyping. The risk of cross-contamination in the PCR laboratory is also reduced [33]. However, it should be noted that when conducting quantitative PCR analysis, differences in amplification efficiency can lead to a significant error, therefore, with the spread of quantitative analysis, the requirements for the purity of the DNA preparations are increasing. Thence, a complete abandonment of simpler methods based on traditional PCR-RFLP seems impractical [33].

Im this works, we genotyped the Russian population of Aberdeen Angus cattle for the myostatin, leptin and calpain 1 genes using DNA tests developed by us. It was shown that the animals contain both wild-type and mutant alleles associated with the productivity performance of beef cattle. For the F94L *MSTN* polymorphism, almost all animals were homozygous for the wild-type allele. For the Arg4Cys *LEP* and CAPN1_316 polymorphisms, wild-type alleles occurred with a high frequency.

The literature data analysis allows us to conclude that the effect of F94L *MSTN* polymorphism is similar to the mutation that causes the genetic defect of double muscularity (M1), but without affecting bodyweight at birth [13-15]. Allele *A* is associated with 5.5% larger silverside area (a valuable part of the carcass) and 2.3% larger areas of the *longissimus dorsi* muscle cross sections at 10-11 ribs, last rib, or between the first and second lumbar vertebrae [13]. In turn, the latterare indirectly associated with increased meat content in the carcass [13].

The phenotypic effects of the F94L *MSTN A* allele were partially recessive. Therefore, the *AA* F94L *MSTN* genotype may produce an intermediate (non-double) muscle phenotype, which is valuble for beef cattle breeding [13]. Therefore, this SNP is considered a very promising genetic marker of meat productivity performance [13-15].

For the Arg4Cys *LEP* polymorphism, it is difficult to definitively indicate which allele is more preferable for selection. On the one hand, the mutant *T* allele associated with higher leptin expression determines better weight gain and fattening qualities which probably explains the high bodyweight of young Aberdeen Angus cattle in our study. On the other hand, the *C* allele is associated with leaner meats which may be beneficial in dietary nutrition [22].

Our data on the frequency of the *T* allele are in lines with other reports. The *T* allele in the population we studied turned out to be relatively frequent and amounted to 0.49-0.51, depending on the animal sex. In the study of Aberdeen

Angus population by F.C. Buchanan et al. [23], this figure was 0.58, being higher compared to the Charolais (0.34), Hereford (0.55) and Simmental (0.32) breeds. The research team of N.P. Gerasimov [19], like us, revealed a significant proportion of heterozygous animals. The *CT* genotype frequency was 50.0% in young animals and 53.3% in adults of the breeding stock. *CT* Arg4Cys *LEP* heifers stood out in terms of meat productivity and showed a tendency to increase pre-slaughter weight by 1.67-2.63% and carcass weight by 0.76-0.85%)compared to the *CC* and *TT* genotypes [19]. In contrast to our findings for the perios from birth to 8-month age, the *TT* Arg4Cys *LEP* cows had minimal productivity parameters [19], however, in the post-weaning period, a similar trend was observed in animals from the Peasant Happy Farm.

For the *CAPN1*_316 polymorphism, a clear idea has already been formed that the *C* allele, associated with increased tenderness of meat, is more preferable for breeding [23, 25, 26]. In our work, when studying the *CAPN1*_316 polymorphism, a significantly larger DNA fragment was amplified. Sanger sequencing of the resulting PCR product revealed the nucleotide sequence NM_174259.2. In this case, there is a possibility of alternative splicing which does not result in excision of non-coding intronic sequences [34, 35]), apparently determining the larger size of the amplified region. This fact requires further study to assess the productive qualities of animals. Our results are of interest, showing the positive effect of the *TT* Arg4Cys *LEP* genotype on the cattle weight gain from birth to 8 months.

It is known that the bodyweight of young animals at the age of 205 days is a characteristic of the milk production of mother cows [36]. However, we cannot exclude the influence of the calves' own genotypes on their bodyweight. Thus, it was found that in carriers of the *CC* genotype for the leptin gene, the feeling of satiety occurs later and the digestibility of feed is higher, which ultimately determines greater bodyweight [17, 21, 22]. Apparently, our data also indicate the influence of the Arg4Cys *LEP* genotype on the average daily weigh gain in calves from birth to weaning.

The study of the *CAPN1*_316 polymorphism led to contradictory results. The *CC CAPN1*_316 heifers aged 6 months had a statistically significantly greater weight compared to heifers with the *GG* genotype, and at the age of 12 months, this trend persisted. Howeverm the *CC CAPN1*_316 bulls at the age of 12 months were inferior in weight to their *GG* peers. E. Casas et al. [25] and S.N. White et al. [26] also did not find a dependence of fattening traits on the genotype for *CAPN1*_316. Probably, this polymorphism has a greater effect on the meat quality parameters, and in order to identify the desired genotype of the Aberdeen Angus breed for *CAPN1*_316, further study of slaughter parameters, in particular the histological and physical-technological characteristics of muscle tissue, is necessary.

The change in the influence of the Arg4Cys *LEP T* allele on the average daily weight gain from 8 to 15 months remains controversial. To explain such differences in fattening performance, in our opinion, the role of leptin as a hormone with multiple effects should be considered. Leptin is involved in the regulation of eating behavior, affects feed digestibility, reproductive and immune functions [37]. It is believed that leptin expression and blood levels are subject to circadian rhythms and vary depending on feeding conditions [37]. Leptin transmits signals through specific leptin receptors (LepR) of two types, the expressed and secreted, which are located on the membranes of target cells. The secreted receptors competitively bind, reducing its interaction with expressed isoforms and, thereof, the effects leptin mediates [38]. In humans, the importance of this mechanism has been established [39]. It ensures changes in the bioavailability of leptin,

especially in the first years of life. Before the onset of puberty, the concentration of soluble LepR continuously decreases [39]. In our opinion, in cattle, as they reach sexual maturity, the main function of leptin becomes the regulation of reproductive qualities and the immune system. Further research is required to verify this assumption. In this regard, the report by M. Zhang et al. [40] is of interest. In their work, a correlation analysis of complex genotypes and the manifestation of economically valuable traits in foxes revealed the interaction between the genes of leptin and its receptor with a synergistic effect [40].

Continuing research will provide tools for genotyping cattle populations for productivity polymorphisms and identifying genetic markers that have the strongest associations with desirable traits. This will help improve the predictive accuracy of genomic selection and accelerate selective improvement in herds [41, 42]. To increase the profitability of beef cattle breeding, the selection of young animals with better fattening potential is also promising.

Thus, on an Aberdeen Angus cattle population as an example, we developed test systems for DNA diagnostics of the Arg4Cys *LEP* and *CAPN1*_316 polymorphisms and performed beef cattle genotyping to select carriers of desired genotypes. Among the animals tested, *CC* F94L *MSTN*, *TC* Arg4Cys *LEP* and *GG* *CAPN1*_316 genotypes were the most common. For rapid fattening of animals under the age of one year, the *TT* Arg4Cys *LEP* genotype is desireable due to a significant superiority to peers in terms of average daily weight gain. The DNA tests we have developed require inexpensive and readily available reagents. This simplifies testing in molecular genetic laboratories to identify individuals with the desired genotypes for productivity.

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