Qualitative and quantitative assessment of beef productivity in Aberdeen-Angus cows and heifers depending on the C73T/C528T haplotype in leptin gene

A N Ruchay¹, ², ³, K M Dzhulamanov¹, N P Gerasimov¹ and V I Kolpakov¹

¹Federal Research Centre of Biological Systems and Agro-technologies of the Russian Academy of Sciences, 29, 9 Yanvarya Str., Orenburg, 460000, Russia
²Department of Mathematics, Chelyabinsk State University, Bratiev Kashirinykh 129, Chelyabinsk 454001, Russia
³South Ural State University (National Research University), Lenin prospekt 76, Chelyabinsk 454080, Russia

E-mail: nick.gerasimov@rambler.ru

Abstract. Special attention is paid to polymorphic variants of leptin gene when organizing MAS selection in beef cattle breeding. In this regard, the purpose of our research was a quantitative and qualitative assessment of meat productivity in Aberdeen-Angus cows and heifers depending on the C73T / C528T haplotype of leptin gene. The study focused on cows after the first calving (age 32 months, n = 30) and heifers (age 20 months, n = 49) of Aberdeen-Angus breed. The data analysis showed that the rank of distribution did not repeat across the age categories of carriers by weight of chilled carcasses in particular combinations. The best quality carcasses were obtained from heifers with a combination of TT / CC, CT / CT and CC / CT genotypes. The highest categories (Prime and Top Choice) were 70.0; 69.2 and 66.6% of carcasses, respectively. Genetic variability in leptin gene in the second exon (C73T) and promoter (C528T) regions in Aberdeen-Angus cows and heifers determined the differences in meat productivity. The combination of heterozygous CT / CT genotypes was recognized as the most preferred for selection in mature herd for a complex of traits of quantitative and qualitative assessment of meat productivity.

1. Introduction
Genetic variability in leptin gene was first described in humans and mice [1, 2]. Subsequently, changes in the nucleotide sequence of leptin gene were revealed in many farm animals, which made it possible to establish a link between polymorphisms and development of a wide range of economically useful qualities [3, 4, 5]. The C73T polymorphism (Arg25Cys, R25C or 305C> T) occurs in 2 gene axon in 25 codon with functional replacement of arginine with cysteine, resulting in the N-terminal signal peptide loss, and, thus, leptin cannot be secreted from adipose tissue [6]. This mutation is associated with fat content in carcass, in particular, the synthesis of cysteine is associated with high fat content, and arginine with leaner ones in beef cattle [7]. The effect of Arg25Cys polymorphism on the mass and quality of carcasses was assessed when raising bull-calves and heifers. Wherein, there is a positive trend of TT genotype to the carcass category according to Canadian classification, which was explained by the peculiarities of fat formation in carriers of different alleles. On the contrary, a large carcasses weight...
was noted in individuals with CC genotype [8]. The change in nucleotide sequence at LEP C528T position is much less involved in MAS selection against the background of the widespread use of C73T polymorphism in the second exon of leptin in breeding work with cattle. This single nucleotide polymorphism occurs in the 5'-untranslated region of leptin gene promoter [9]. The LEP C528T nucleotide substitution is associated with a significant increase in blood leptin concentration by 39-48% in carriers of TT genotype compared to peers with CT and CC genotypes, respectively. Кроме того, мутация в промоторной области C528T гена лептина связана повышенным на 31-39 % синтезом подкожной жировой клетчатки, оценки «мраморности» мяса на 9-13 % [10]. In addition, a mutation in promoter region C528T (UASMS2) of leptin gene is associated with an increased synthesis of subcutaneous adipose tissue by 31-39%, and meat “marbling” by 9-13% [10].

Thus, special attention is paid to polymorphic variants of leptin gene when organizing MAS selection in beef cattle breeding. In this regard, the purpose of our research was a quantitative and qualitative assessment of meat productivity in Aberdeen-Angus cows and heifers depending on the C73T / C528T haplotype of the leptin gene.

2. Materials and methods
The study focused on cows after the first calving (age 32 months, n = 30) and heifers (age 20 months, n = 49) of Aberdeen-Angus breed.

To determine the polymorphisms C73T and C528T in leptin gene, blood samples were taken from the jugular vein in experimental animals (Blood samples were taken from the jugular vein in experimental animals to determine the polymorphisms C73T and C528T in leptin gene). Blood was pumped into tubes with 600 μL of ethylenediamine tetraacetic acid (EDTA) to obtain 10 ml volume. DNA samples were extracted from whole blood using a Sample-HS reagents set (DNA technology, Russia). The quality of the isolated DNA was evaluated using horizontal electrophoresis "SE-2" (Helicon, Russia) in 1.5% agarose gel "Agarose, Biotechnology grade" (Helicon, Russia). Bio-Rad CFX 96 (Bio-rad, USA) and a ready-made mixture qPCRmix-HS (Evrogen, Russia) were used to carry out the polymerase chain reaction. The primers were synthesized at the Scientific and Production Company "Litekh" (table 1).

### Table 1. Nucleotide sequences and conditions for amplification of the studied polymorphic regions of GH, TG5, LEP genes.

| SNP       | Nucleotide sequence                                                                 | References |
|-----------|--------------------------------------------------------------------------------------|------------|
| LEP C73T  | F: 5'-ggaccccctgtwtcgattcct-3’<br>R: 5’-tgtcttgatgagggttttgg-3’                    | [7]        |
| LEP C528T | F: 5’-aggtgcccagggactca-3’<br>R: 5’-caacaaaggccgtgtgaca-3’                         | [10]       |

PCR protocol: initial warming up to +37 °C for 5 min; denaturation at +94 °C for 5 min; further 40 cycles: +94 ° C - 15 sec; +60 ° C - 1 min.

Control slaughter of experimental animals was carried out according to GOST R 54315-2011 “Cattle for slaughter. Beef and veal in carcasses, half carcasses and quarters ”. Carcasses were classified according to American standards [11]. Определение мраморности мясной продукции – по развитию внутримышечного жира на срезе длиннейшей мышцы спины между 12 и 13 ребрами [12]. Marbling of meat products was determined by the development of intramuscular fat on a section of rib eye between the 12th and 13th ribs [12].

Data were processed by the methods of basic statistics using the Microsoft Office “Excel 10.0” and the specialized program “Statistica 10” (“Stat Soft Inc.”, USA). The statistical difference between the mean values were assessed by the a posteriori method "Tukey's test for unequal N". Values at P≤0.05 were considered reliable.
3. Results

Various combinations of C73T and C528T polymorphisms of leptin gene affected the differences in cooled carcass weight in cows and heifers (table 2). Wherein, the distribution rank of particular combinations was not repeated according to the age categories of bearers. The greatest chilled carcasses weight was observed in heifers-carriers of TT / CT complex, which exceeded their peers by 4.4-26.7 (1.37-8.90%; P > 0.05). At the same time, cows with this haplotype of leptin gene were lower by 7.7 kg (2.40%) than the average expression of the trait among their peers. Also, the inverse distribution rank was found in cows and heifers with a CT / TT combination.

Table 2. Carcass characteristic of Aberdeen Angus heifers and with different haplotypes for leptin gene (LEP C73T and LEP C528T), (X±Sx).

| SNP       | Heifers | Cows |
|-----------|---------|------|
| LEP C73T  | LEP C528T | n  | Cooled carcass weight, kg | n  | Cooled carcass weight, kg |
| CT        | CT      | 13  | 315.3±6.45               | 11  | 314.0±19.28               |
| CC        | CT      | 6   | 315.2±10.94              | 1   | 407.9                     |
| TT        | CC      | 10  | 304.3±5.19               | 8   | 316.9±15.85              |
| CC        | TT      | 10  | 310.9±4.68               | 2   | 307.5±87.85               |
| TT        | CT      | 5   | 326.6±6.10               | 3   | 313.3±18.94              |
| CC        | CC      | 1   | 300.1                   | -   | -                        |
| CT        | TT      | 2   | 299.9±3.65               | 1   | 351.5                     |
| CT        | CC      | 2   | 322.2±21.10              | 4   | 331.3±31.47              |
| Average   |         | 49  | 312.7±2.81               | 30  | 321.0±10.42              |

The CT / CC haplotype was more stable in chilled carcass weight in the context of the studied age categories of animals. The bearers of this combination exhibited high meat productivity regardless of the control slaughter age. Thus, heifers with CT / CC genotype exceeded their average peers by 9.5 kg (3.04%), and cows had a 10.3 kg (3.21%) advantage. The minimum chilled carcass weight was revealed for cows with a CC / TT combination (307.5 kg). At the same time, the productivity of heifers with a similar leptin gene combination was also below average.

Genotyping of animals taking into account polymorphisms C528T and C73T of leptin gene showed uneven distribution of carcasses by categories depending on the haplotype (figure 1, 2).

Figure 1. Carcass distribution by category in Aberdeen-Angus heifers with different leptin gene haplotypes (LEP C73T and LEP C528T).
The best quality carcasses were obtained from heifers with a combination of TT / CC, CT / CT and CC / CT genotypes. The highest categories (Prime and Top Choice) were 70.0; 69.2 and 66.6% of carcasses, respectively. It should be noted that the massiveness of carcasses did not ensure the high quality of meat raw materials. Thus, the carcasses in the category “SELECT” had the largest weight (326.9 kg), with an advantage over the ratings of “Prime” and “Top Choice” by 9.3-16.5 kg (2.93-5.32%). According to above-mentioned, the most productive CT / CC and TT / CT combined genotypes of heifers were characterized by relatively low carcass quality. Thus, the intense weight growth of Aberdeen-Angus young cattle has a negative effect on the intramuscular fat deposition and "marbling" meat raw materials.

A similar trend was found in the controlled slaughter of cows. Thus, carriers of the CT / CT haplotype had better carcass quality. Wherein, the “Select” rating was assigned to 45.4% of carcasses. During the slaughter of cows, the superiority of the heterozygous haplotype carriers was confirmed in terms of the quality of meat raw materials.

4. Conclusion
Genetic variability in leptin gene in the second exon (C73T) and promoter (C528T) regions in Aberdeen-Angus cows and heifers determined the differences in meat productivity. The combination of heterozygous CT / CT genotypes was recognized as the most preferred (preferable) for selection in mature herd for a complex of traits of quantitative and qualitative assessment of meat productivity.

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References
[1] Ohshiro Y, Ueda K, Nishi M, Ishigame M, Wakasaki H, Kawashima H, Furuta H, Sasaki H, Sanke T, Takasu N and Nanjo K 2000 A polymorphic marker in the Leptin gene associated with Japanese morbid obesity J. Molecular Medicine 78 516-20
[2] Halaas J L, Gajiwala K S, Maffei M, Cohen S L, Chait B T, Rabinowitz D, Lallone R L, Burley S K and Friedman J M 1995 Weight reducing effects of the plasma protein encoded by the obese Genetic Science 269 543-6
[3] Giblin L, Stephen T B, Kearney B M, Waters S M, Callanan M J and Berry D P 2010 Association
of bovine leptin polymorphisms with energy output and energy storage traits in progeny tested Holstein-Friesian dairy cattle sires. *BMC Genetics* **11** 73 doi:10.1186/1471-2156-11-73

[4] Sadeghi S, Hajihosseinlo A and Bohlouli M 2014 Haplotype association of ovine leptin gene on breeding value of body measurements in Makooei sheep breed *Biotechnology in Animal Husbandry* **30(2)** 233-42 doi:10.2298/BAH1402233S

[5] Sedykh T A, Kalashnikova L A, Gusev I V, Pavlova I Y, Gizatullin R S and Dolmatova I Y 2016 Influence of TG5 and LEP gene polymorphism on quantitative and qualitative meat composition in beef calves *Iraqi Journal of Veterinary Sciences* **30(2)** 41-8 DOI:10.33899/jvvs.2016.121382

[6] Zhang Y, Proenca R, Maffei M, Barone M, Leopold L and Friedman J M 1994 Positional cloning of the mouse obese gene and its human homologue *Nature* **372** 425-32

[7] Buchanan F C, Fitzsimmons C J, Van Kessel A G, Thue T D, Winkelman-Sim D C and Schmutz S M 2002 Association of a missense mutation in the bovine leptin gene with carcass fat content and leptin mRNA levels *Genet Sel Evol* **34(1)** 105-16 doi:10.1051/gse:2001006

[8] Kononoff P J, Deobald H M, Stewart E L, Laycock A D and Marquess F L S 2005 The effect of a leptin single nucleotide polymorphism on quality grade, yield grade, and carcass weight of beef cattle *J Anim Sci* **83(4)** 927-32 doi: 10.2527/2005.834927x

[9] Larionova P V 2006 *Development and experimental testing of systems for the analysis of polymorphism of candidate genes for lipid metabolism in cattle* (Dubrovitsy)

[10] Nkrumah J D, Li C, Yu J, Hansen C, Keisler D H and Moore S S 2005 Polymorphism in the bovine leptin gene promoter associated with serum leptin concentration, growth, feed intake, feeding behavior, and measures of carcass merit *J Anim Sci* **83(1)** 20-8 doi:10.2527/2005.83120x

[11] Hale D S, Goodson K and Savell J W 2013 *USDA Beef Quality and Yield Grades* (Department of Animal Science, Texas A&M AgriLife Extension Service College Station)

[12] United States Standards for Grades of Carcass Beef 2017 (United States Department of Agriculture)