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## DEVELOPING MEAT PRODUCTIVITY IN BULL CALVES OF DIFFERENT DGAT1 GENOTYPES

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The given **research aims** to study the way meat productivity in special-purpose beef bull calves of different DGAT1 genotypes is developed. The **scientific novelty** of the research lies in the fact that an assessment of the meat productivity of Hereford and Limousin bulls of different DGAT1 genotypes was conducted for the first time. Calves were cultivated using elements of resource-saving technology. The **research subject** was Hereford male young stock (91 heads) and Limousin bull calves (109 heads), which were genotyped by SNP DGAT1-K232A. Live weight, average daily gains, and body size and conformation indices were **analyzed**. Hematological values and carcass quality of bull calves of different genotypes were **studied**. As a result of genotyping, young animals of both breeds had a similar distribution of genotypes ( $DGAT1^{KK} > DGAT1^{KA} > DGAT1^{AA}$ ) and alleles ( $DGAT1^K > DGAT1^A$ ). There was no effect of the studied gene polymorphism on growth, body development, and hematological parameters, as bull calves of different DGAT1 genotypes did not show a significant difference between weight and linear growth, blood morphological parameters, the content of protein, and its fractions. SNP DGAT1-K232A was found to affect fat deposition. Thus, carcasses of both studied breeds of  $DGAT1^K$  genotype had a significantly higher content of internal raw fat, and fat yield was ( $P < 0.05$ ) than carcasses of  $DGAT1^{AA}$  genotype bull calves. Therefore, genotyping by SNP DGAT1-K232A can be used in the selection of special-purpose beef cattle as an additional criterion to produce meat of a higher energy value.

**Keywords:** hereford breed; limousin breed; genotype; DGAT1; fattening qualities; meat qualities; development in postnatal ontogenesis

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## ФОРМИРОВАНИЕ МЯСНОЙ ПРОДУКТИВНОСТИ У БЫЧКОВ РАЗНЫХ ГЕНОТИПОВ DGAT1

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*Целью данного исследования* является изучение того, как развивается мясная продуктивность у мясных бычков специального назначения различных генотипов DGAT1. **Научная новизна** исследований заключается в том, что впервые проведена оценка мясной продуктивности бычков герефордской и лимузинской пород различных генотипов по гену DGAT1, выращивание которых проводилось с использованием элементов ресурсосберегающей технологии. **Объектом исследования** были бычки герефордской породы (91 голова) и бычки лимузинской породы (109 голов), которые генотипировались по SNP DGAT1-K232A. Были **проанализированы** живая масса, среднесуточные приросты, а также размеры тела и показатели телосложения. **Изучены** гематологические показатели и качество туши бычков разных генотипов. В результате генотипирования молодые животные обеих пород имели сходное распределение генотипов (DGAT1<sup>KK</sup>>DGAT1<sup>KA</sup>>DGAT1<sup>AA</sup>) и аллелей (DGAT1<sup>K</sup>>DGAT1<sup>A</sup>). В ходе исследования нами не было выявлено влияние изучаемого полиморфизма гена на рост, развитие тела и гематологические показатели, так как у бычков разных генотипов DGAT1 не наблюдалось существенной разницы между массой тела и линейным ростом, морфологическими показателями крови, содержанием белка и его фракций. Обнаружено, что SNP DGAT1-K232A влияет на отложение жира. Таким образом, туши обеих изученных пород генотипа DGAT1<sup>K</sup> имели значительно более высокое содержание внутреннего сырого жира, а выход жира был ( $P<0,05$ ), чем у туш бычков генотипа DGAT1<sup>AA</sup>. Следовательно, генотипирование по гену SNP DGAT1-K232A может быть использовано при отборе специализированного мясного скота в качестве дополнительного критерия для получения мяса с более высокой энергетической ценностью.

**Ключевые слова:** герефордская порода; лимузинская порода; генотип; DGAT1; способность к откорму; качество мяса; развитие в постнатальном онтогенезе

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## Introduction

High-quality beef production from special-purpose beef cattle is one of the main growth areas for the Russian meat industry. The beef cattle industry in the Russian Federation is actively developing at present [5; 8]. As stated by the Russian Ministry of Agriculture, the number of cattle in the country amounted to more than 18 million heads in 2018, including special-purpose animals in all categories of farms that reached 2.26 million heads. A comprehensive assessment of 711.16 thousand heads, or 34.1% of the total number of beef cattle, including 389.8 thousand animals of 15 breeds and types bred in 57 regions of the Russian Federation, has shown that the largest populations are Aberdeen-Angus (417,545 heads), Kalmyk (137,262 heads), Hereford (87,278 heads), and Kazakh white-headed breeds (52,563 heads). The controlled livestock is purebred and IV generation, including 99.7% servicing bulls and 99.3% cows. As of January 1, 2019, the breeding base of beef cattle breeding of the country is represented by 270 breeding herds, including 46 breeding plants and 224 breeding reproducers [7].

Under current import substitution and food security strategies in the production of meat for processing, the gene pool of existing cattle breed populations of the Russian selection has received growing academic interest. The research results are used to develop programs for breed conservation and design organic production systems based on domestic genetic resources for selection [5; 7; 21].

The basis of selection and breeding programs is the early and accurate identification of breeding traits of the animal. The advantage of marker selection is the unrelatedness of genetic markers on paratypic factors; they do not change throughout the life of the animal. The mapping of quantitative trait loci (QTL) is of particular relevance for assessing genetic parameters. Additionally, there are difficulties recognizing markers for economic traits due to the low heritability level and trait polygenicity. The quantitative level of traits can be determined genetically by different allele variations in different genome loci. Alleles are concurrently tested. The identified allele variations can be used as markers for individual segments of closely linked chromosomes and genes. Some of these genes can be responsible for productivity traits [6].

Presently, marker selection contributes much to the development of beef cattle breeding and serves as an additional criterion for animal selection, in particular high-value species. It is an important resource for creating highly productive beef herds [1; 17]. Besides, assessing animals by genes that control productive, reproductive, and other economic traits is the most critical indicator of the pedigree livestock [4; 15; 17; 21].

Numerous studies aimed at establishing the relationship between meat productivity traits and single nucleotide polymorphism (*SNP*) of candidate genes are still relevant [6; 8; 9; 11; 14; 18].

Among the genes responsible for lipid metabolism in animals, the diacylglycerol O-acyltransferase 1 gene (*DGATI*) is an enzyme known for synthesizing triglycerides, diglycerides, and acyl-coenzyme A. The *DGATI* gene in cattle was mapped on the BTA14 chromosome. A. Winter et al. [19] have tested the *SNP* in exon 8 of the *DGATI* gene and revealed that the changes  $G \rightarrow A$  and  $C \rightarrow A$  at position 10434 result in the loss of the restriction site for *Cfr*I endonuclease and the replacement (*K232A* polymorphism) of lysine with alanine ( $K \rightarrow A$ ). It has also been found that the lysine-coding allele (*K* allele) is associated with a higher fat content in milk [19]. I. F. Gorlov et al. [4] indicate that *DGATI*<sup>A</sup> is more common among *Bos taurus taurus* and practically not found among *Bos taurus indicus* and *Bos grunniens*.

Some Russian and foreign researchers claim that the polymorphism of the diacylglycerol-O-acyltransferase 1 (*DGATI*) gene is associated with the fatness of carcasses and the quality of meat [9; 11]. B. M. Sørensen et al. [18] have found a high correlation of the *DGATI* enzyme activity in cattle with the fat content in the longissimus muscle and the eye round. Research by R. A. Curi et al. [10] conducted on the Nelore cattle has shown that the *K* allele *DGATI* positively affects the subcutaneous fat layer thickness [10]. I. Anton et al. [8] have determined the effect of the *DGATI* gene polymorphism on the intramuscular fat content in Hungarian Angus. X. X. Wu et al. [20] consider that *DGATI* is a promising genetic marker for intramuscular fat content (*IMF*). L. Pannier et al. [14] have explored a significant impact of the marker on the subcutaneous fat content in the Charolais and Limousin metapopulations. It should be noted that studies of the *DGATI* polymorphism effect on meat productivity and quality traits of *Bos* animals failed to observe a reliable impact.

In our previous studies on genotyping Hereford and Limousin bull calves by the *DGATI* gene [16; 17], there were no resulting animals with the homozygous genotype by the second allele. In this regard, it was decided to increase the sample of experimental animals and the list of studied traits characterizing the development of meat qualities in young beef livestock in postnatal ontogenesis.

The scientific novelty of the research lies in the fact that an assessment of the meat productivity of Hereford and Limousin bulls of different *DGATI* genotypes was conducted for the first time. Calves were cultivated using elements of resource-saving technology.

## Materials and Methods

The research aimed to study the development of meat productivity in bull calves of various *DGAT1* genotypes. The research tasks were as follows: (1) genotyping Hereford and Limousin bull calves according to *SNP DGAT1-K232A*; (2) assessing live weight, average daily gain, and body size and conformational parameters of animals aged eight and twenty months; and (3) studying hematological parameters and quality of carcass of bull calves of various genotypes.

For genotyping by *SNP DGAT1-K232A*, Hereford (91 heads) and Limousin (109 heads) bull calves were genotyped at the age of one month. The young Hereford bulls were the offspring of the Australian selection animals bred in the livestock breeding farm SAVA-Argo-Usen LLC; Limousin bull calves descended from animals obtained in the livestock breeding farm SAVA-Agro-Yapryk LLC (Tuymazinsky district of the Republic of Bashkortostan, Russia) by accumulation crossbreeding of Simmental cattle with bulls of the French selection. The farms use stable-pasture technology, and summer maintenance on pasture is conducted according to the *cow-calf* system with resource-saving elements: (1) using empty livestock premises and ground runs for keeping cows with calves; (2) arranging rounded calving with regulated suckling; and (3) maximum use of pasture lands with electric fences and fattening of commercial young animals on open year-round operated feedlots. Bull calves were reared up to twenty months of age [2].

DNA was isolated from whole blood stabilized with sodium citrate using a set of *DNA-Extran (Syntol)*. Genotyping was performed by the PCR-RFLP method [9] using primers: F: 5'-gca-cca-tcc-tct-tcc-TCA-ag-3' and R: 5'-gga-agc-gct-ttc-tcc-gga-tg-3'. Amplifiers were cleaved by the *CfiI* endonuclease. The number and length of the received restriction fragments were found electrophoretically in 7.5% PAGE in UV light after staining with ethidium bromide. Restriction fragments were analyzed using the Gel Doc XR gel documentation system with the Image Lab version 2.0 *DNA-analyser* software. Restriction fragment sizes were as follows: 411 KK, 411, 208, 203 KA, and 208, 203 AA base pairs.

Depending on the identified genotypes, bull calves were divided into groups by the *DGAT1* gene: Group I – *DGAT1<sup>KK</sup>* (n=20); Group II – *DGAT1<sup>KA</sup>* (n=20); Group III – *DGAT1<sup>AA</sup>* (n=10).

The development of meat productivity was studied according to the dynamic indicators of live weight, average daily gains, conformation measurements, body-built indices, hematological values, and post-slaughter assessment of the carcass quality.

The live weight of calves was determined by weighing them on a floor scale at birth, eight months, twelve months, sixteen months, and twenty months. The average daily gain was found by the ratio of overall live weight gain for the growing period to the number of days in the period. Conformation recording was conducted at eight and twenty months of age, and standard and linear measurements were studied, namely, withers height, chest girth, chest width, chest depth, oblique body length, pastern width, and quarter size. Based on these measurements, conformation indices were calculated, particularly shoulder width, boniness, blockiness, frame size, meatiness, and body length index.

Hematological studies were performed under the clinical diagnostic laboratory. The blood morphological composition was determined by flow cytometry on an automatic LH-500 analyzer (Beckman Coulter). The total protein content was evaluated using an SYNCHRON CX4 PRO biochemical analyzer (Beckman Coulter). Protein fraction content was identified by the capillary electrophoresis automated solution MINICAP (Sebia).

Post-slaughter carcass evaluation of different genotype bull calves was performed at the SAVA meat processing plant. The carcass quality was assessed according to the Russian national standard GOST 33818-2016 – *Meat. High-quality beef. Specifications*. Pre-slaughter live weight, hot carcass weight, carcass yield, the weight of internal raw fat, fat yield, slaughter weight, and slaughter yield were found according to the classical methods developed in the All-Russian Animal Breeding Institute named after L. K. Ernst.

Statistical processing of the research results was conducted by standard methods using the software application *Microsoft Office Excel*.

## Results

Genotyping Hereford and Limousin bull calves revealed a high frequency of occurrence of the  $DGATI^{KK}$  genotype, which was 51.65% and 50.46%, being higher than that of  $DGATI^{KA}$  by 16.49% and 11.93%, and  $DGATI^{AA}$  by 38.46% and 39.45%, respectively. In general, bull calves of both studied breeds are characterized by the same frequencies of the  $DGATI^K$  and  $DGATI^A$  alleles.

The live weight dynamics of different genotype animals by the  $DGATI$  gene are shown in Fig. 1.

The analysis of the obtained data on the dynamics of the live weight of Hereford and Limousin animals shows that there is no effect of the  $DGATI$  gene polymorphism on the live weight indicators of calves at birth and eight, twelve, sixteen, and twenty months of age. There is a tendency of a certain increase in the bodyweight of animals in the direction of  $DGATI^{KK} \rightarrow DGATI^{KA} \rightarrow DGATI^{AA}$ .

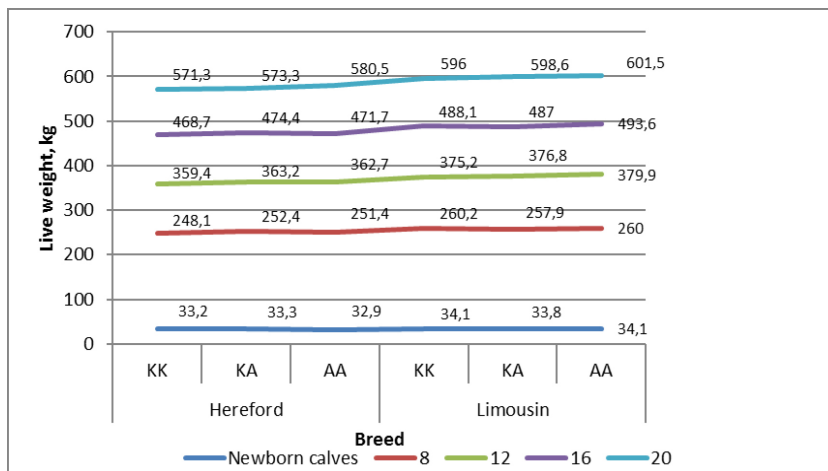


Fig. 1. Live weight dynamics of different genotype bull calves by the *DGATI* gene, kg.

With increasing age,  $DGATI^{KK} < DGATI^{AA}$  genotype Hereford bull calves showed a slight difference in live weight: 0.91% at the age of twelve months, 0.65% at sixteen months, and 1.58% at twenty months; Limousin animals had 1.25%, 1.13%, and 0.91%, respectively. It should be noted that the live weight results are within the standards for the Hereford and Limousin breeds.

Compared to Hereford cattle, Limousin animals had slightly higher live weight indicators in all age periods – by 3.37% at eight months, 4.11% at twelve months, 3.67% at sixteen months, and 3.95% at twenty months.

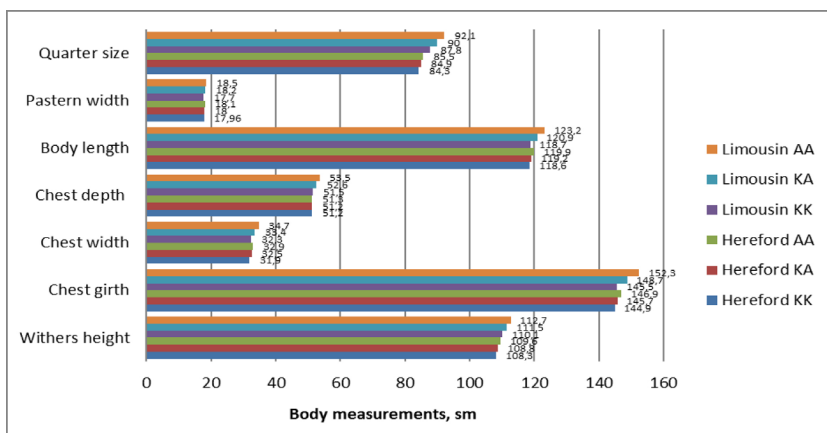
The average daily gain in live weight decreases with the increasing age of animals.  $DGATI^{AA}$  and  $DGATI^{KA}$  genotype Hereford bull calves showed the highest average daily gains for the entire growing period (912.7 g and 900.0 g, respectively). Limousin bulls of the identical genotypes had 945.7 g and 941.3 g of average daily weight gain.

The blood morphological composition of different genotype animals did not have significant differences and was within the reference limits of the physiological standards. Haemoglobin, red blood cells, and white blood cell contents at eight months were slightly higher than at twenty months, which is associated with age characteristics.  $DGATI^{AA}$  genotype bull calves of both breeds had a relatively higher level of red blood cells and hemoglobin.

The concentration of protein fractions was also within the range. The results show some age-related features. Thus,  $DGATI^{KK}$  genotype Hereford and

Limousin young bulls had a higher amount of total protein by an average of 11.47% and 12.04% by twenty months of age, *DGATI<sup>KA</sup>* genotype animals – by 12.07% and 10.12%, and *DGATI<sup>AA</sup>* genotype cattle – by 9.19% and 10.54%, respectively. *DGATI<sup>AA</sup>* genotype bull calves of both breeds had more albumins and  $\gamma$ -globulins at the age of eight months, with a higher level of gamma-globulins at twenty months of age. Hereford cattle showed a slight increase in the protein coefficient (albumin-globulin index).

Conformation measurements of different *DGATI* genotype bull calves are shown in Fig. 2 and 3.



**Fig. 2.** Conformation measurements of calves at the age of eight months.

The received confirmation measurements of Hereford and Limousin bull calves – withers height, chest girth, chest width, chest depth, oblique body length, pastern width, and quarter size – were within the age norms and norms of the breed standard. Bull calves of different genotypes did not have apparent dissimilarities between the indicators. However, *DGATI<sup>AA</sup>* genotype bull calves had slightly higher conformation measurements at the age of eight months and twenty months, which indicates a better body development in young animals and is confirmed by higher live weight indicators.

In the age aspect, Hereford bull calves had increased withers height by 15.48%, chest girth by 31.34%, chest width by 51.40%, chest depth by 43.47%, pastern width by 27.45%, oblique body length by 18.59%, and quarter size by 44.40%; those of Limousin cattle were higher by 15.90%, 33.47%, 50.83%, 43.94%, 27.04%, 16.73%, and 42.31%, respectively.



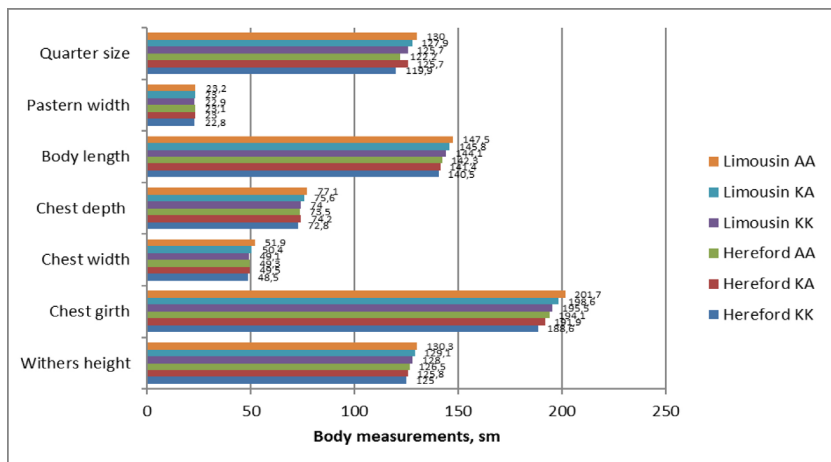


Fig. 3. Conformation measurements of calves at the age of twenty months.

Body-built indices of different genotype bull calves are given in Fig. 4 and 5.

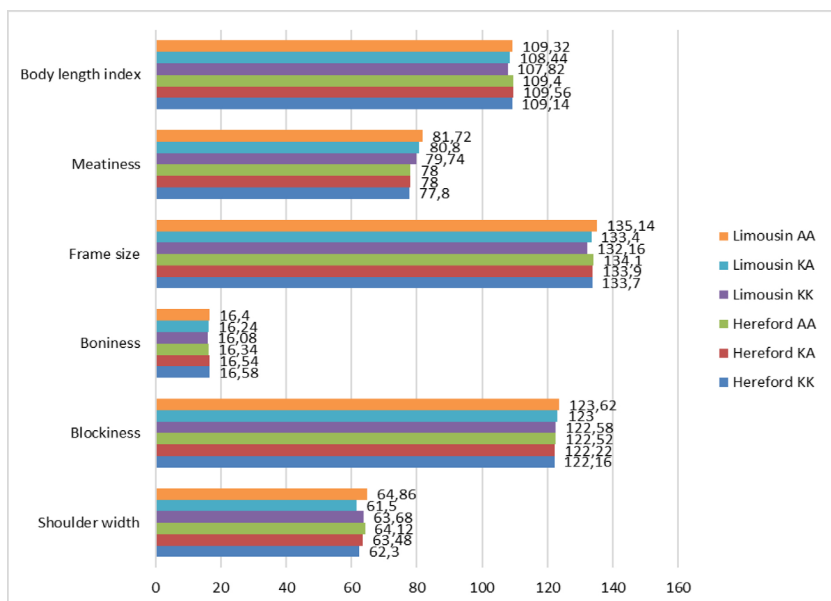


Fig. 4. Body-built indices of calves at the age of eight months.

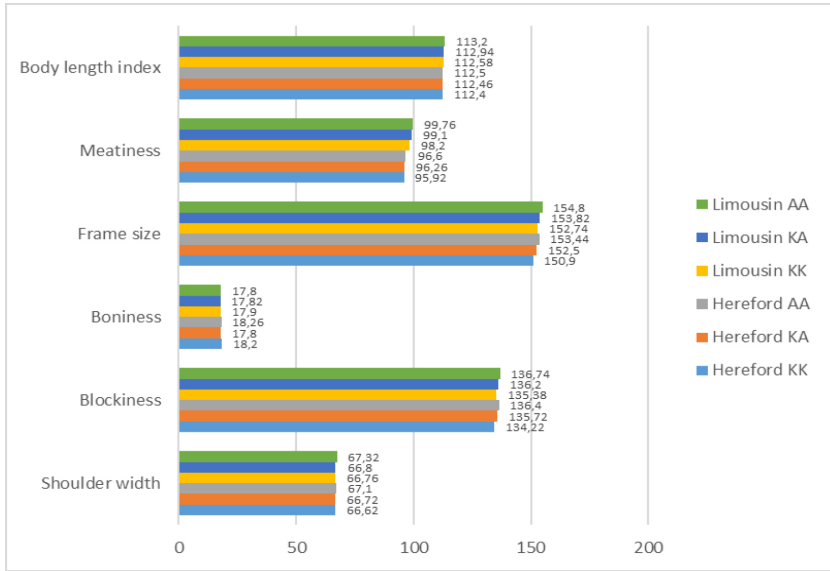


Fig. 5. Body-built indices of calves at the age of twenty months.

The increase of body-built indices, including those characterizing the development of meat quality indicators observed with age, demonstrates the harmonious development of the body parts of both breeds and confirms the distinct beef breed manifestation of young animals in all experimental groups.

In the context of genotypes, there is a tendency for a slight increase in meat productivity indices in the direction of  $DGATI^{KK} \rightarrow DGATI^{KA} \rightarrow DGATI^{AA}$ . At the age of eight months,  $DGATI^{AA}$  genotype Hereford and Limousin animals had a slight increase in shoulder width by 1.82% and 1.18%, frame size by 0.4% and 2.98%, and meatiness by 0.2% and 1.98%, respectively, compared with the young animals of the  $DGATI^{KK}$  genotype. At the age of twenty months, Hereford and Limousin bull calves had a similar difference: 0.48% and 0.56% in the shoulder width, 2.54% and 2.06% in the frame size, and 0.68% and 1.56% in meatiness, respectively. Young animals at the age of eight months were characterized by a more compact build, while at the age of twenty months, the body length index increased. This was especially noticeable in the cattle of the Limousin breed characterized by a long body. In general, the studied indicators allowed us to conclude the harmonious body development of both studied breeds.

Slaughter results of different *DGAT1* genotype bull calves are shown in Fig. 6 and 7.

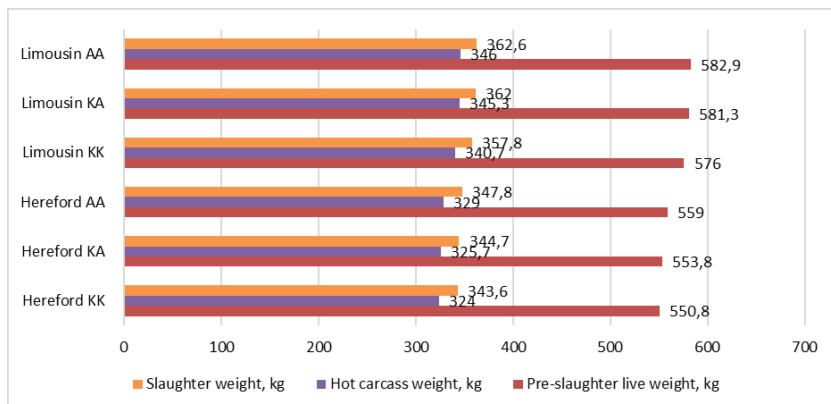


Fig. 6. Carcass quality indicators of different *DGAT1* gene genotype bull calves.

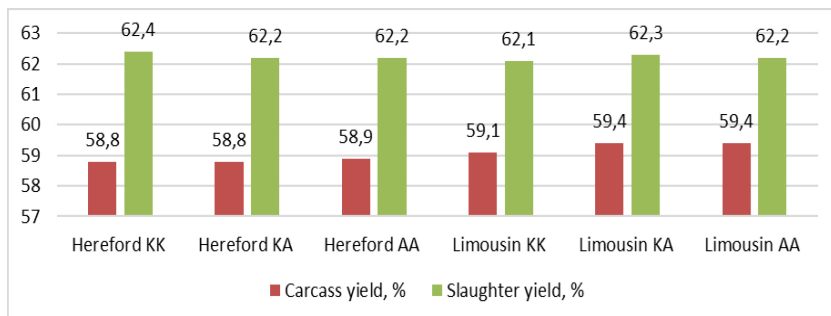


Fig. 7. Carcass and slaughter yield of different *DGAT1* gene genotype bull calves.

Carcass quality indicators of different *DGAT1* gene genotype bull calves demonstrate the absence of intergroup differences. There is a tendency to increase them somewhat  $DGAT1^{KK} \rightarrow DGAT1^{KA} \rightarrow DGAT1^{AA}$ . Indicators of pre-slaughter live weight in Hereford and Limousin cattle with the  $DGAT1^{AA}$  genotype are higher by 1.47% and 1.19% compared to animals with the  $DGAT1^{KK}$  genotype. In studying animal carcasses, the difference  $DGAT1^{AA} > DGAT1^{KK}$  is also observed, in the order of listing breeds, respectively: hot carcass weight by 1.52% and 1.53%, carcass yield by 0.01% and 0.30%, slaughter weight by 1.21% and 1.32%, and slaughter yield by 0.20%.

There is a clear association of the studied *DGATI* gene polymorphism with fat content in both breeds. Thus, there were significant differences ( $P < 0.05$ ) between the *DGATI<sup>KK</sup>* > *DGATI<sup>AA</sup>* genotypes in terms of the internal raw fat weight (19.6 kg and 18.75 kg in Herefords; 17.12 kg and 16.63 kg in Limousins) and fat yield (3.6% and 3.35%; 3.0% and 2.85%, respectively).

**Discussion.** The findings of genotyping Hereford and Limousin bull calves indicate a high *DGATI<sup>KK</sup>* genotype frequency of 50%–51% in both breeds. The heterozygous genotype is in second place in terms of frequency, and the smallest number of animals were of the homozygous genotype by the second allele. In general, bull calves of both studied breeds are characterized by the same frequencies of the *DGATI<sup>K</sup>* (0.64) and *DGATI<sup>A</sup>* (0.36) alleles. The observed frequencies of the *DGATI<sup>K</sup>* allele (0.64) are slightly lower than the findings of C. Avilés et al. [9] that have revealed allele frequencies in Spanish Limousins as follows: *DGATI<sup>K</sup>* (0.84) and *DGATI<sup>A</sup>* (0.18). There is evidence of the *DGATI<sup>KK</sup>* genotype absence in the Simmental bull calves [4] and the Kazakh white-headed animals [3].

The research did not prove associations of the studied polymorphism with growth and development indicators of bull calves. Hence, there were no significant inter-group differences in terms of live weight, average daily gains, absolute live weight gains, hematological values, conformation and body-built measurements, and body composition indices. The results of the control slaughter proved a strong association of the *DGATI* gene polymorphism in the bulls of both breeds with the fat content. The *DGATI<sup>K</sup>* genotype was associated with the internal raw fat weight and the fat yield. The findings of the given research are consistent with the results of J. L. Gill et al. [7], C. Avilés et al. [9], and A. Curi et al. [10], who indicated increased fat deposition in *DGATI<sup>KK</sup>* genotype bull calves. This dependence was not found in other studies [10; 13]. The obtained results go in line with D. Karolyi, who found that *DGATI<sup>AA</sup>* genotype bull calves exceeded *DGATI<sup>KA</sup>* animals in carcass weight by 2.09% [12].

**Conclusion.** Thus, in the conditions of the Republic of Bashkortostan, Hereford and Limousin beef cattle were genotyped by *SNP DGATI-K232A*. This gene polymorphism was studied in terms of its effect on the meat productivity of young animals in postnatal ontogenesis. As a result of genotyping, young animals of both breeds proved to have a similar distribution of genotypes (*DGATI<sup>KK</sup>* > *DGATI<sup>KA</sup>* > *DGATI<sup>AA</sup>*) and alleles (*DGATI<sup>K</sup>* > *DGATI<sup>A</sup>*). There was no effect of the studied gene polymorphism on growth, body development, and hematological parameters, as bull calves of different *DGATI* genotypes did not show a significant difference between weight and linear growth, blood morphological

parameters, the content of protein, its fractions, and carcass quality. *SNP DGAT1-K232A* was found to affect fat deposition. Thus, carcasses of both studied breeds of *DGAT1<sup>K</sup>* genotype had a significantly higher content of internal raw fat, and fat yield was ( $P < 0.05$ ) than carcasses of *DGAT1<sup>AA</sup>* genotype bull calves. Therefore, genotyping by *SNP DGAT1-K232A* can be used as an additional criterion to get meat with a higher energy value in selecting special-purpose beef cattle.

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