

Influence of TG5 and LEP gene polymorphism on quantitative and qualitative meat composition in beef calves

T.A. Sedykh*¹, L.A. Kalashnikova², I.V. Gusev³, I.Yu. Pavlova², R.S. Gizatullin¹
and I.Yu. Dolmatova¹

¹Bashkir State Agrarian University, 50-letiya Oktyabrya str., 34, Ufa, Republic of Bashkortostan, 450001, ²Russian Research Institute of Stock Breeding, Lenin str., Lesnye, Pushkinskii district, Moscow Region, 141212, ³L.K. Ernst Russian Research Institute of Animal Breeding, Dubrovicy, Podol'skii district, Moscow Region 142132
Corresponding author: editor3@academicpapers.org, nio_bsau@mail.ru.

(Received September 13, 2016; Accepted November 27, 2016)

Abstract

The authors investigated the influence of TG5 and LEP gene polymorphism on quantitative and qualitative meat composition of 20 month old bull calves of the Hereford (n=38) and Limousine (n=26) breeds, which were bred in the climate of Cis-Ural steppe zone from 2013 to 2015. The Hereford calves were the offspring of the cattle from the southeastern states of Australia and Tasmania (3rd descent of the main lines: Baz-Gol-Sol 2U 6827, Domino 325676, Ardmors-Domino 56, Silverlend 31432); the Limousine calves were the descendants of the offspring resulting from the accumulation cross breeding of Simmental cattle with the French Reproductive Recognized bulls (4th descent of the Reper 433 and other lines). The analysis of TG5 genotype frequency in the examined populations reveals that the animals have significant (P<0.01 or P<0.05) potential for increased taste and nutritional qualities of beef associated with a high proportion of desirable TT genotype, probably related to the foreign origin of the cattle. There were no carriers of the BB genotype of LEP genes in the examined populations. Significant (P<0.01 or P<0.05) dependence between the studied SNP in TG5 and the rates of total body fat, the proportion of adipose tissue in the morphological carcass composition (or meat composition), and the content of intramuscular fat in the longissimus, as well as the correlation between the studied SNP in LEP and the rates of raw visceral fat, and fat outcome, were established. LEP polymorphism was significantly (P<0.01 or P<0.05) associated with the proportion of adipose tissue in the morphological carcass composition in Hereford calves, and with the content of intramuscular fat in the longissimus in Limousine calves. The results of our study of TG5 and LEP polymorphism demonstrate considerable genetic potential of the given populations of the Hereford and Limousine breeds in relation to the quantitative and qualitative composition of their meat; they are to be used in improvement of genetic potential of meat cattle in the Cis-Ural steppe zone.

Keywords: Meat productivity, Polymorphism, TG5, LEP, Hereford calves, Limousine calves
Available online at <http://www.vetmedmosul.org/ijvs>

علاقة الجين تي جي ٥ و ولب جين متعدد الاشكال على نوعية وكمية مكونات اللحم في عجول التسمين

سدياكيه تي اي، كلاشنيكوفه ال اي، كوسيف اي في، بافلوفا اي يو، كستاليون ار اس، دولماتوف اي يو

الجامعة الزراعية في بشهكار، جمهورية بيشكورستان، المعهد الروسي للتنمية الحيوانية، موسكو

الخلاصة

تم دراسة العلاقة الجينية للجين تيجي ٥ و واللب جين متعدد الاشكال على كمية ونوعية لحم عجول بعمر ٢٠ شهر. استخدم في الدراسة عجول كانت ولدت في مناخ من رابطة الدول المستقلة الأورال وكانت منطقة السهوب من عام ٢٠١٣ إلى عام ٢٠١٥. والعجول هيريفورد نسل الماشية من دول جنوب شرق أستراليا وتسمانيا (أصل 3rd من الخطوط الرئيسية: الباز غول سول 2U 6827، دومينو

325676، Ardmors-دومينو 56، Silverlend 31432)؛ في حين كانت العجول ليموزين من نسل ذرية الناتجة عن تربية تراكم عبر الأبقار Simmental مع الثيران الفرنسية الإنجابية المعترف بها (أصل 4th من Reper 433 وخطوط أخرى. أظهرت نتائج تحليل التردد الوراثي ان الجين تيحي لديه علاقة معنوية ($P < 0.01$ أو $P < 0.05$) في إمكانية زيادة الذوق وصفات غذائية في لحوم البقر يرتبط مع نسبة عالية من النمط الجيني TT مرغوب فيه، وربما كانت متصلة أصل أجنبي من الماشية. لم تكن هناك شراكات من النمط الجيني BB الجينات LEP في TG5 ومعدلات إجمالي الدهون في الجسم.

Introduction

Nowadays the proportion of the beef cattle population in Russia equals to 3%, whereas in the countries with efficient beef-cattle industry it comes up to 40-80% (1,2). It should be noted that over the 2009 to 2014 period the development of specialized beef farming in Russia has been intensified: the population of purebred and crossbred cattle has come over 2 million head, and the beef proportion has reached 12.7% (1). In the Republic of Bashkortostan beef production mostly depends on the of young cattle (young calves or calves), which is not used for replacement, and adult cull cows of Black Pied, Simmental, Bestuzhev, and some other breeds. During the last five years (2011-2015) the proportion of specialized beef cattle, including Hereford, Limousine, Aberdeen Angus and Simmental breeds, has increased due to the implementation of several federal and regional programs, and is now equal to more than 8% (2). Meat of beef cattle is of high taste quality due to the content of intramuscular fat (marbling appearance) and the moisture-retaining capacity, which define the color, juiciness, flavor and tenderness of the product. The combination of these characteristics determines demand and thus the price of the product. The content of subcutaneous fat in a carcass determines its quality after postmortem chilling, provides beef fermentation and optimal ageing duration, which is important for the food processing industry. Beef quality depends on the animal breed, its genetic potential, housing and feeding (the main important factors) conditions, slaughtering age, storage and packaging techniques (3-8). The methods of genetics enable us to determine genes responsible for the productive qualities. Identification of alleles of such genes gives the possibility of additional direct selection at the DNA level along with traditional selection, which can enhance the genetic potential of the animals and promote successful development of the industry (9-12). The relevance of investigation of the associations between animal meat productivity and single nucleotide polymorphisms (SNP) in candidate genes has been repeatedly justified by the results of multiple studies (13-17). The leptin gene (LEP) and thyroglobulin (TG5) gene are directly involved in lipid metabolism. Leptin is a hormone produced by adipocytes, the fat cells, and it plays a key role in metabolism, particularly in fat accumulation, it is involved in feeding behavior (or quantity and quality of food) regulation, it

influences the immune system functioning and reproduction, as well as height and body type or size of an animal. In cattle, LEP is located in chromosome 4q32 region. It consists of three exons and two introns, and only two exons are translated into the protein (18). There are about 60 SNPs described in LEP (17) (Yoon *et al.*, 2005), the most extensively studied polymorphisms R25C and Y7F are located on the second, and A80V – on the third exon of LEP (19-21), within the region between the second and the third exons (22). The thyroglobulin gene is supposed to be a candidate gene for QTL, influencing fat accumulation and beef marbling in cattle. TG5 is located in the centromere region of the 14 chromosomes (BTA14), it is encoding approximately 8.7 kb RNA and it is considered one of the most eukaryotic genes, as the whole locus length exceeds 200 kb (23). C422T mutation takes place in the 5'-promoter region of the gene (24). C→T substitution in the position 422 of the thyroglobulin gene promotes the emergence of two allelic variations, five more SNPs were discovered with the help of amplification of the gene regions and PCR product sequencing (25). This research was aimed at studying the influence of the TG5 and LEP polymorphism in meat cattle on the quantitative and qualitative composition of beef. The research objectives included (1) calculating the frequencies of the genotypes and alleles of the examined SNP genes; (2) estimating the real and expected heterozygosity, heterozygote excess and deficit; (3) revealing the influence of the TG5 and LEP genes polymorphism on meat productivity, morphologic composition of carcass and chemical composition of the longissimus muscle in postmortem examination. Certain genetic potential has been accumulated within the republic for the moment, basing on the gene pool of imported cattle of foreign selection (Australia, France) (26). Rational use of this potential will allow creation of valuable breeding herds, whose further breeding will provide the increase in commercially viable high-quality meat production.

Materials and methods

Animals

20 month old bull calves, bred in the Cis-Ural steppe zone, were genotyped by TG5 and LEP genes. Hereford (n=38) and Limousine (n=26) breeds, which were bred in the climate of the Cis-Ural steppe zone from 2013 to 2015. The Hereford calves were the offspring of the cattle from

the southeastern states of Australia (3rd descent of the main lines: Baz-Gol-Sol 2U 6827, Domino 325676, Ardmors-Domino 56, Silverlend 31432); the Limousine calves were the descendants of the offspring resulting from the accumulation cross breeding of Simmental cattle with the French Reproductive Recognized bulls (4th descent of the Reper 433 and other lines). Both farms are located in Tuimazy district of the Republic of Bashkortostan (Cis-Ural steppe zone) and perform stable-grazing keeping of beef cattle including resource conservation techniques (27). Depending on the set of genotypes of the steers of each breed, by the method of analogues in live weight and development groups were formed with different combinations of alleles of the genes studied TG5 and LEP: homozygous for allele 1 - 11 - I of the group; heterozygous - 12 - II of the group; homozygous for the allele 2 - 22 - III of the group. The period of growing calves was about 20 months.

Sample collection and phenotyping

Final live weight and pre-slaughter live weight (Tables 3 and 4) were estimated in live animals to assess meat productivity. The post mortem examination was performed at the *SAVA* meat processing factory, including measurements of hot carcass weight and carcass yield, raw visceral fat weight and fat yield, dressing weight and slaughter yield. Morphological composition of carcasses was determined during the dissection of natural anatomic parts of beef sides and designing. Sides were refrigerated according to existing regulations. Samples for the analysis of chemical composition of the longissimus were collected between 11 and 13 ribs. (1). The samples were frozen and transferred to the division of biochemical and analytical research of L. K. Ernst Russian Research Institute of Animal Breeding. Determination of physicochemical parameters included chemical analysis of muscle tissue according to GOST 23042-86: Meat and meat products. Methods of fat determination; GOST R 53642-2009: Meat and meat products. Determination of total ash; GOST 25011-81: Meat and meat products. Methods of protein determination. GOST R 51479-99: Meat and meat products. Method for determination of moisture content.

DNA extraction and genotyping

The analysis was performed at the DNA-technologies laboratories of the Russian Research Institute of Stock Breeding and Molecular Genetics of Bashkir State Agrarian University. DNA extraction was performed according to the standard procedure. TG5 and LEP genes were analyzed by polymerase chain reaction (PCR) and restriction fragment length polymorphism (RFLP) methods. Following primers were used for gene fragments amplification: TG5:1 – 5'-ggg-gat-gac-tac-gag-tat-gac-tg-3; TG5:2 - 5'-ctg-aaa-atc-ttg-tgg-agg-ctg-ta-3 (28)(De et al., 2004). LEP:1 - 5'-tgg-

agt-ggc-ttg-tta-ttt-tct-tct-3; LEP:1 – 5'-gtc-ccc-gct-tct-ggc-tac-cta-act-3. This structure can be explained by shape (22). During PCR (30-35 cycles), annealing temperatures equaled 60 °C for TG5 and 65 °C for LEP. The amplicates of TG5 and LEP genes were restricted by BStx2L and Sau3AL endonucleases respectively. The number and lengths of the restriction fragments were determined by 3%-agarose gel electrophoresis, ethidium bromide staining and UV-visualization, then analyzed by the means of computing system and gel-documentation. The frequencies of separate alleles and genotypes were determined with the use of traditional methods (29).

Statistical analysis

Expected heterozygosity rate (H_e) was calculated from the results of genotyping the calves by DNA-markers, as follows: $H_e = 1 - \sum p_i^2$ (where p_i is the frequency of the i^{th} allele). Observed heterozygosity rate (H_o) was calculated as the ratio of heterozygous to the total amount of animals examined: $H_o = n/N$ (where n is the number of animals, heterozygous for the given allele, N is the sampling size). χ^2 was used to evaluate the correlation between observed and expected distributions of the genotypes within the examined sample sets, which was calculated as

$$\chi^2 = \sum (O - E)^2 / E$$

(where O and E are observed and expected numbers respectively of certain genotypes, k is the number of genotypic categories). Statistical analysis of the data was conducted using the STATISTICA 5.0 software specially designed for processing statistical data.

Results

The data on TG5 and LEP gene polymorphism in Hereford and Limousine calves are given in Table 1. It was shown that there is no significant difference in frequencies of TG5 genotypes between Hereford and Limousine calves – CC (45% and 52%), CT (39.5% and 38.5%), TT (7.9% and 15.3%). The BB genotype of LEP gene is completely absent. In general, the unwanted AA genotype is prevalent in Hereford calves (68.4%), whereas in Limousine calves conditionally useful AA genotype is dominant (61.5%). Observed and expected heterozygosity rates are given in Table 2. The excessive proportion of TG5 and LEP heterozygotes is observed in Hereford calves. In Limousine calves there is deficiency in TG5 heterozygotes and significant excess (0,187) in LEP heterozygotes. It should be noted that the Hereford population is balanced by TG5, and the value of the correlation criterion of observed and expected distributions of LEP genotypes is higher in Limousine calves. It can result from either small sampling size or alterations in the gene pool due to accumulation cross breeding. The most important requirement for raw

meat is its high qualitative characteristics and biological usefulness, which are based on smart growth and

development of the animal tissues.

Table 1. TG5 and LEP genes polymorphism

Breed	n	Genotypes						Allelic frequencies	
		CC		CT		TT		C	T
TG5		Head No.	%	Head No.	%	Head No.	%		
Hereford	38	20	52.6	15	39.5	3	7.9	0.75	0.25
Limousine	26	12	46.2	10	38.5	4	15.3	0.65	0.35
LEP		AA		AB		BB		A	B
		Head	%	Head	%	Head	%		
Hereford	38	26	68.4	12	31.6	0	0	0.84	0.16
Limousine	26	10	38.5	16	61.5	0	0	0.69	0.31

Table 2. Observed and expected TG5 and LEP heterozygosity rates

Breed	H _o	H _e	F	χ ²
TG5				
Hereford	0.395	0.374	0.021	0.12
Limousine	0.385	0.454	-0.069	1.05
LEP				
Hereford	0.316	0.268	0.048	0.86
Limousine	0.615	0.428	0.187	8.17

H_o observed heterozygosity; H_e expected heterozygosity; F Ho-He discrepancy, «+/-» excess/deficiency in heterozygotes, χ² criterion of correlation between observed and expected distributions of genotypes.

Tables 3 and 4 contain the results of the postmortem evaluation of meat productivity. The data in Table 3 indicate that in the course of investigation of TG5 and LEP genes polymorphism influence significant (P<0.01 or P<0.05) association was only observed for the raw visceral fat weight and fat output. Concerning TG5 gene, fat output in the carcasses of both breeds was significantly increased in TT genotype (3.64%), exceeding CC genotype by 0.2-0.3% (P<0.01) and CT genotype by 0.1% (P<0.05). The calves with CC genotype tend to have higher values of pre-slaughter live weight, hot carcass weight, carcass yield, dressing weight and slaughter yield. In course of investigating the connection between LEP gene polymorphism and the results of postmortem assessment of meat productivity (Table 4), two parameters were found to be significantly correlated. The carcasses of Hereford and Limousine calves of AB genotype had significantly higher (P<0.01) raw visceral fat weight (19.2% and 16.2%) than those of the AA genotype (18.6% and 16.9%) by an average 0.65%, and higher fat yield (P<0.05) by an average 0.18%.

Tables 5 and 6 sum up the morphological composition of the carcasses of calves with different TG5 and LEP genotypes. The results of the analysis of morphological composition of the carcasses indicate that the values of fat content in the carcasses of both breeds depend on the TG5 gene polymorphism. The carcasses of Hereford calves of different genotypes have higher proportion of more fat content (6.59-6.67-6.91%) than those of Limousine calves (5.97-6.35-6.39), which can be explained by breed characteristics, as Hereford cattle tend to have a more effective fat gain. The carcasses of Hereford calves of TT, TC and CC genotypes have significantly different (P<0.05) fat content with the difference equal to 0.32%, whereas in those of Limousine calves the difference equals to 0.38 and 0.42%. The animals of the CC genotype of both breeds tend to have slightly higher values of chilled carcass yield and proportion of meat, bones and tendons. The fat proportion in the morphological composition of the genotypes. Carcasses of LEP heterozygous Hereford calves is significantly increased (P<0.05) by 0.62%, whereas in Limousine calves the value of fat proportion tends to be higher in AB genotype (by 0.19%). Meatiness coefficient is higher in Limousine calves of different TG5 and LEP genotypes compared to Hereford calves, which is the result of the increased ability of this breed to build up muscle tissue. The data on the chemical composition of the longissimus are summed up in tables 7 and 8. The data of the chemical composition of the longissimus indicates the association of intramuscular fat content in the longissimus and TG5 polymorphism in Hereford and Limousine calves, as there is a significant (P<0.05) difference of 0.68 and 0.72% between TT and CC.

The calves of the TT genotype tend to have a lower proportion of total moisture or dry matter and higher proportion of dry matter. The Limousine calves of AB genotype of LEP gene have significantly increased (P<0.05) proportion of intramuscular fat in the longissimus by 0.78%.

Table 3. Results of postmortem assessment of meat productivity of the calves with different TG5 genotypes, $X \pm S_x$

Characteristic	Breed/genotype					
	Hereford (n=13)			Limousine (n=13)		
	CC (n=5)	CT (n=5)	TT (n=3)	CC (n=5)	CT (n=5)	TT (n=3)
Live weight in the end of growth and sagination, kg	576.2±14.93	568.6±14.47	554.9±17.53	604.2±16.14	590.5±15.87	588.3±14.9
Pre-slaughter live weight, kg	560.6±13.30	550.3±13.20	538.0±13.09	585.1±13.06	572.8±13.08	570.0±12.81
Hot carcass weight, kg	333.6±12.48	325.8±12.41	316.9±12.39	356.9±12.42	347.1±12.31	342.6±12.29
Carcass yield, %	59.5±1.14	59.2±1.12	58.9±1.10	61.0±1.14	60.6±1.16	60.10±1.12
Raw visceral fat weight, kg	19.4±0.70	19.5±0.73	19.6±0.80	17.2±0.56	17.6±0.58	18.0±0.60
Fat yield, %	3.46±0.02	3.54±0.02*	3.64±0.02**	2.84±0.06	3.08±0.06*	3.14±0.14**
Dressing weight, kg	353.0±13.15	345.4±13.08	336.5±13.05	374.1±12.98	364.7±12.84	360.6±12.82
Slaughter yield, %	62.9±1.25	62.7±1.23	62.5±1.19	63.9±1.18	63.6±1.21	63.2±1.20

* P<0.05; ** P<0.

Table 4. Results of postmortem assessment of meat productivity of the calves with different LEP genotypes, $X \pm S_x$

Characteristic	Breed/genotype			
	Hereford (n=13)		Limousine (n=13)	
	AA(n=8)	AB(n=5)	AA(n=4)	AB(n=9)
Live weight in the end of growth and sagination, kg	580.4±15.35	566.3±14.83	599.7±15.39	584.5±15.42
Pre-slaughter live weight, kg	560.1±13.28	549.5±13.92*	577.8±13.16	565.6±13.72
Hot carcass weight, kg	331.6±12.69	323.1±12.71	350.7±12.62	341.0±12.58
Carcass yield, %	59.2±1.15	58.8±1.13	60.7±1.14	60.3±1.10
Raw visceral fat weight, kg	18.6±0.22	19.2±0.29**	16.2±0.30	16.9±0.34**
Fat yield, %	3.32±0.04	3.50±0.05*	2.81±0.04	2.99±0.03*
Dressing weight, kg	350.2±13.18	342.3±13.06	366.9±13.15	357.9±12.90
Slaughter yield, %	62.5±1.24	62.3±1.25	63.4±1.21	63.2±1.19

* P<0.05; ** P<0.01.

Table 5. Morphological composition of the carcasses of calves with different TG5 genotypes, ($X \pm S_x$)

Characteristic	Breed/genotype					
	Hereford			Limousine		
	CC (n=5)	CT (n=5)	TT (n=3)	CC (n=5)	CT (n=5)	TT (n=3)
Chilled side weight, kg	164.92±6.35	160.84±4.11	155.55±5.83	175.20±6.42	170.00±5.31	167.77±5.41
including meat, kg	120.68±4.45	117.93±4.44	113.62±4.28	130.78±4.01	126.10±4.82	124.36±4.15
%	73.17±2.15	73.26±2.15	73.09±2.08	74.65±2.66	74.18±2.86	74.13±2.45
fat, kg	10.86±0.38	10.73±0.32	10.74±0.29	10.46±0.36	10.80±0.22	10.72±0.31
%	6.59±0.08	6.67±0.12	6.91±0.09*	5.97±0.11	6.35±0.12*	6.39±0.13*
bones, kg	28.47±0.81	27.71±0.95	26.74±0.84	29.10±0.92	28.40±0.79	28.04±0.81
%	17.26±0.45	17.21±0.42	17.20±0.44	16.61±0.51	16.71±0.45	16.71±0.43
tendons and cartilages, kg	4.91±0.33	4.50±0.31	4.45±0.33	4.86±0.34	4.70±0.32	4.65±0.35
%	2.98±0.09	2.80±0.07	2.86±0.09	2.77±0.07	2.76±0.08	2.77±0.08
Meatness coefficient	4.24±0.12	4.26±0.09	4.25±0.08	4.49±0.13	4.44±0.12	4.44±0.10

*P<0.05.

Table 6. Morphological composition of the carcasses of calves with different LEP genotypes, ($X \pm S_x$)

Characteristic	Breed/genotype			
	Hereford		Limousine	
	AA(n=8)	AB(n=5)	AA(n=4)	AB(n=9)
Chilled side weight, kg	163.48±7.12	162.28±6.43	172.32±6.88	166.58±6.49
including meat, kg	119.79±4.35	118.00±4.42	127.60±4.61	122.99±4.55
%	73.28±2.31	72.71±2.15	74.05±2.42	73.83±2.39
fat, kg	10.85±0.41	11.78±0.35	11.02±0.31	10.97±0.35
%	6.64±0.18	7.26±0.17*	6.40±0.14	6.59±0.17
bones, kg	28.13±0.92	27.86±0.93	28.90±0.86	28.00±0.94
%	17.21±0.51	17.17±0.46	16.77±0.45	16.81±0.48
tendons and cartilages, kg	4.71±0.31	4.64±0.30	4.80±0.35	4.62±0.32
%	2.88±0.07	2.86±0.08	2.79±0.09	2.77±0.07
Meatness coefficient	4.26±0.12	4.24±0.11	4.42±0.15	4.39±0.13

*P<0.05.

Table 7. Chemical composition of the longissimus in calves with different TG5 genotypes, ($X \pm S_x$)

Characteristic	Breed/genotype					
	Hereford			Limousine		
	CC (n=5)	CT (n=5)	TT (n=3)	CC (n=5)	CT (n=5)	TT (n=3)
Total moisture, %	72.32±0.81	71.73±1.05	71.08±0.97	72.70±1.12	72.25±0.99	72.10±1.14
Dry matter, %	27.68±0.49	28.27±0.61	28.92±0.55	27.30±0.45	27.75±0.71	27.90±0.89
including protein	20.94±1.07	21.29±0.93	21.47±0.27	20.49±0.39	20.87±0.42	20.38±0.30
fat	5.78±0.18	6.01±0.25	6.47±0.22*	5.84±0.40	5.91±0.41	6.56±0.24*
ash	0.96±0.04	0.97±0.03	0.98±0.03	0.97±0.03	0.97±0.03	0.96±0.02
Phosphorus, g/kg	1.17±0.03	1.24±0.03	1.18±0.02	1.15±0.03	1.15±0.02	1.15±0.03

*P<0.05.

Table 8. Chemical composition of the longissimus in calves with different LEP genotypes, ($X \pm S_x$)

Characteristic	Breed/genotype			
	Hereford		Limousine	
	AA(n=5)	AB(n=5)	AA(n=5)	AB(n=5)
Total moisture, %	72.01±1.02	71.00±0.86	72.70±1.07	71.28±0.94
Dry matter, %	27.90±0.78	29.00±0.49	27.30±0.61	28.72±0.89
including protein	20.95±0.50	21.44±0.37	20.49±0.42	20.86±0.55
fat	5.98±0.21	6.62±0.28	5.84±0.34	6.62±0.27*
ash	0.96±0.05	0.94±0.07	0.97±0.06	0.94±0.05
Phosphorus, g/kg	1.19±0.02	1.20±0.03	1.15±0.02	1.13±0.03

*P<0.05.

Discussion

It has been shown that the frequencies of TG5 genotypes do not differ significantly in Hereford and Limousine calves, whereas AA genotype of LEP is dominant in Hereford calves (68.4%) and AB genotype is prevalent in Limousine calves (61.5%), which is consistent with the previous research by different authors, showing the breed specificity in gene proportions, which can be

explained by the influence of other genes of the same cluster on the gene of interest, repressing or altering its effect on the phenotype 14-16, 22 (4,5,8). The calves with the BB genotype of LEP are absent in the populations, which can result from insufficient sampling size for this kind of research or from commercial breeding aimed at the increase in live weight of the animals without regard to quality of meat. Similar research concerning the Hereford cattle population of Siberia shows that 65-76% of cattle

have CC genotype, the frequency of heterozygous genotype is 20-35% and more, whereas only one herd included the carriers of desirable TT genotype (2%) (14-16). The data obtained by the Tatar Agriculture Research Institute experts (13). Indicate the absence of Hereford cattle with TT genotype, whereas in Limousine cattle the frequency of TT genotype equaled to 22.6% (7 head). The occurrence of conditionally useful CT genotype in Hereford cattle was 29% (9 head) and 11.1% (2 head) in Hereford cattle. Thus the data on frequency of different TG5 genotypes indicates significant potential of cattle of both breeds occurring in the republic concerning taste and nutritional qualities of meat due to relatively high proportion of the animals with desirable TT genotype, which can be explained by the foreign origin of the meat cattle.

Leptin and thyroglobulin gene polymorphisms were not associated with the investigated characteristics of growth rates of the animals. The data on live weight dynamics are consistent with the results of V.A. (14), who did not observe significant influence of genotypes on the growth rate in calves.

In the course of investigating the influence of the C422T SNP in TG5 gene, we observed the tendency to higher values of pre-slaughter live weight, hot carcass yield, carcass output, dressing weight and slaughter yield in CC genotype cattle. Similarly, comparable research by T.D. Carvalho *et al.* (30) has not shown a significant association between TG5 polymorphism and meat productivity characteristics, although the authors observed the tendency to higher pre-slaughter live weight in TT genotype cattle (which contradicts our results) and increased thickness of subcutaneous fat in CC genotype cattle. Our data on fat yield is completely consistent with the results by Casas *et al.* (25), who have shown the increase in fat yield in TT genotype cattle.

There is a certain controversy in the results of research concerning the association of different SNPs in LEP gene with quantitative and qualitative characteristics of beef (30).

Carvalho *et al.* (30), Gill *et al.* (25), Schenkel *et al.* (31) Aviles *et al.* (3), have shown a significant association between LEP gene polymorphism and the thickness of subcutaneous fat, CC genotype cattle having the lowest values of this parameter, which indirectly supports our results. However, other researchers, including Curi *et al.* (4) have observed no such correlation in case of LEP/BsaAI SNPs.

We have shown significant ($p < 0.01$ and $p < 0.05$) association of LEP gene polymorphism and raw visceral fat weight and fat yield.

The association between TG5 gene polymorphism and fat proportion in morphological composition carcass, as well as the content of intramuscular fat in the longissimus was observed in Hereford and Limousine calves. These

results are consistent with those by Anton *et al.* (32), Barendse *et al.* (23), Casas *et al.* (25), and not consistent with those by Carvalho *et al.* (30), who have found no significant association between these characteristics and TG5 gene polymorphism.

Conclusions

Competitive meat production supposes improvement of domestic cattle breeds through using the genetic potential of imported breeds in selection. It explains the importance of studying the influence of the TG5 and LEP genes polymorphism on the quality of the Hereford cattle imported from Australia and the Limousine breed obtained as a result of accumulation cross breeding of the local Simmental cattle with French bulls. The research revealed the association of the TG5 SNP gene and fat yield, fat ratio in the morphological composition of carcass and intramuscular fat in the longissimus in calves of both breeds. In both breeds, the influence of the LEP SNP gene on fat yield was determined — in Hereford calves on fat ratio in carcass morphological composition, in Limousine calves — on longissimus fat content. The results of our research concerning TG5 and LEP gene polymorphism indicate high genetic potential in the observed populations of Hereford and Limousine cattle regarding quantitative and qualitative characteristics of the meat. The authors believe that further research is necessary, including larger sampling size and other beef cattle breeds of the region. The results of relevant studies would be applicable for enhancing genetic potential of beef cattle bred in the Cis-Ural steppe zone.

References

1. Legoshin GP, Afanasyeva ES, Mogilenets ON. Effect of pre-slaughter live weight of calves in their productivity and quality of carcasses. *Myasnaya Industriya [Meat Industry]*. 2014;8:54-55 (in Russian).
2. Sedykh TA, Gizatullin RS. Ways to improve the efficiency of beef production in beef cattle. *Fundamentalniye Issledovaniya [Fundamental Research]*, 2015;2(18):3971-3975 (in Russian).
3. Aviles C, Polvillo O, Pena F, Juarez M, Martinez AL, Molina A. Associations between DGAT1, FABP4, LEP, RORC, and SCD1 gene polymorphisms and fat deposition in Spanish commercial beef. *Anim Biotech*. 2015;26(1):40-44.
4. Curi RA, Chardulo LAL, Arrigoni MDB, Silveira AC, Oliveira HN. Associations between LEP, DGAT1 and FABP4 gene polymorphisms and carcass and meat traits in Nelore and crossbred beef cattle. *Livestock Sci*. 2011;135:244-250.
5. Fortes MRS, Curi RA, Chardulo LAL, Silveira AC, Assumpcao MEOD, Visintin JA et al. Bovine gene polymorphisms related to fat deposition and meat tenderness. *Genet Mol Biol*. 2009;32:75-82.
6. Gill JL, Bishop SC, McCorquodale C, Williams JL, Wiener P. Association of selected SNP with carcass and taste panel assessed meat quality traits in a commercial population of Aberdeen Angus-sired beef cattle. *Genetics Selection Evolution*. 2009;41:36.
7. Li X, Ekerljung M, Lundstrom K, Lunden A. Association of polymorphisms at DGAT1, leptin, SCD1, CAPN1 and CAST genes

- with color, marbling and water holding capacity in meat from beef cattle populations in Sweden. *Meat Sci.* 2013;94:153-158.
8. Prevolnik M, Čandek-Potokar M, Škorjanc D. Predicting pork waterholding capacity with NIR spectroscopy in relation to different reference methods. *J Food Eng.* 2010;98:347-352.
 9. Alipanah M, Kalashnikova L., Rodionov G. Association of prolactin gene with milk production traits in Russian red pied cattle. *Iranian J Biotech.* 2007;5(3):158-161.
 10. Zinovieva NA, Kostyunina OV, Gladyr EA et al. The role of DNA markers productivity traits. *Zootehnika [Zootechny].* 2010;1:8-10 (in Russian).
 11. Bega MA, Habibrahmanova YL, Kalashnikova LA, Trufanov VG. Polymorphism of genes of leptin (LEP), thyroglobulin (TG5) and beta-casein (CSN2) from Holstein cows. *Collected Scientific Works of the All-Russian Scientific Research Institute of Sheep and Goat.* 2014;3(7):487-491 (in Russian).
 12. Zinovieva NA, Klenovitsky PM, Gladyr EA, Moseikin LG, Gendzhieva OB. *Biotechnological methods in zootechnics and veterinary.* Elista, Russia. 2014 (in Russian).
 13. Sharipov AA, Shakirov ShK, Yulmeteva LI, Gafurov YR. Molecular aspects of genetically breeding beef cattle for meat marbling. *Vestnik Myasnogo Skotovodstva [Beef Cattle Breeding Bulletin].* 2014;2(85):59-64 (in Russian).
 14. Soloshenko VA, Goncharenko GM, Inerbaev BO, Hramtsova IA, Goryachev TS, Grishina NB. Effect of gene thyroglobulin polymorphism and growth hormone on the growth rate in cattle. *Problemy Biologii Produktivnykh Zhivotnykh [Problems of Productive Animals Biology].* 2011;1:55-58 (in Russian).
 15. Soloshenko VA, Goncharenko GM, Dvoryatkin AA. On the possibility of using genetic markers in breeding beef cattle to improve meat quality indicators. *Vestnik Myasnogo Skotovodstva [Beef Cattle Breeding Bulletin].* 2013;1(17):27-30 (in Russian).
 16. Soloshenko VA, Goncharenko GM, Pleshakov VA, Dvoryatkin AA, Grishina NB, Goryacheva TS. Comparative analysis of beef cattle breeds in Siberia gene TG5 (marbling meat). *Dostizheniya nauki i tekhniki APK [Scientific and Technological Advances in Agriculture].* 2014;1:52-53 (in Russian).
 17. Yoon DH, Cho BH, Park BL *et al.* Highly polymorphic bovine leptin gene. *J Anim Sci.* 2005;18(11):1548-1551.
 18. Kalashnikova LA *et al.* Guidelines for the assessment of genomic cattle. Forest Glade: All-Russian Research Institute of Animal Breeding. 2015 (in Russian).
 19. Szyda J., Komisarek J. Statistical modeling of candidate gene effects on milk production traits in dairy cattle. *J Dairy Sci.* 2007;90:2971-2979.
 20. Komisarek J. Impact of LEP and LEPR gene polymorphism on functional traits in Polish Holstein-Friesian cattle. *Anim Sci Papers Reports.* 2010;10:133-141.
 21. Szyda J, Morek-Kopec M, Komisarek J, Zarnecki A. Evaluation markers in selected genes for association with functional longevity of dairy cattle. *BMC Genetics.* 2011;12:30.
 22. Sharifzadeh A, Doosti A, Moshkelani S. Genetic Polymorphism at the Leptin Gene in Iranian Holstein Cattle by PCR-RFLP. *J Anim Vet Advances.* 2010;9:1420-1422.
 23. Barendse W, Bunch R, Thomas M, Armitage S, Baud S, Donaldson N. The TG5 thyroglobulin gene test for a marbling quantitative trait loci evaluated in feedlot cattle. *Aust J Exp Agric.* 2004;44:669-674.
 24. Hou GY, Yuan ZR, Zhou HL *et al.* Association of thyroglobulin gene variants with carcass and meat quality traits in beef cattle. *Mol Biol Reports.* 2011;38:4705-4708.
 25. Casas E, White SN, Shackelford SD, Wheeler TL, Koohmaraie M, Bennett GL, Smith TPL. Assessing the association of single nucleotide polymorphisms at the thyroglobulin gene with carcass traits in beef cattle. *J Anim Sci.* 2007;12:2807-2814.
 26. Gizatullin RS, Khaziakhmetov FS, Sedykh TA, Mudarisov RM, Khaliullin RG. The production of beef with various technologies for beef cattle. Ufa, Russia: Bashkir State Agrarian University Publishing. 2013.
 27. Sedykh TA, Salikhov AR, Gizatullin RS. The intensity of growth and development of Hereford steers and implementation of meat at different ages. *Izvestiya Orenburgskogo Gosudarstvennogo Agrarnogo Universiteta [Bulletin of Orenburg State Agrarian University].* 2015;2:112-115 (in Russian).
 28. De S, MacNeil MD, Wu XL *et al.* Detection of quantitative trait loci for marbling and backfat in Wagyu × Limousin F2 crosses using a candidate gene approach. *Amer Soc Anim Sci V.* 2004;55:95-98.
 29. Zinovieva NA, Klenovitsky PM, Gladyr EA, Nikishov AA. Modern methods of genetic control of selection processes and Certification of breeding material in animal husbandry. Moscow, Russia: People's Friendship University. 2008. (in Russian).
 30. Carvalho ThD *et al.* Association of polymorphisms in the leptin and thyroglobulin genes with meat quality and carcass traits in beef cattle. *Revista Brasileira de Zootecnia.* 2012;41(10):2162-2168.
 31. Schenkel FS, Miller SP, Ye X, Moore SS, Nkrumah JD, Li C, Yu J, Mandell IB, Wilton JW, Williams JL. Association of single nucleotide polymorphisms in the leptin gene with carcass and meat quality traits of beef cattle. *J Anim Sci.* 2005;11:2009-2020.
 32. Anton I, Kovacs K, Hollo G, Farkas V, Lehel L, Hajda Z, Zsolnai A. Effect of leptin, DGAT1 and TG gene polymorphisms on the intramuscular fat of Angus cattle in Hungary. *Livestock Sci.* 2011;135:300-303.