

The Influence of Growth Hormone Gene Polymorphism on Growth Rate of Young Cattle

Research Article

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ABSTRACT

Beef production is an important development area in animal breeding with meat quality being determined by both paratypic and genetic factors. In this regard evaluating genetic material for the presence of desirable allele combinations of genes associated with growth and development indicators, as well as meat qualities of animals have a certain scientific and practical significance. The aim of the study was to determine the influence of growth hormone gene polymorphism on the growth rate of bull calves of different breeds kept in the Bashkortostan Republic. The method of polymerase chain reaction (PCR) followed by the analysis of restriction fragment length polymorphism (PFLP) (SNP *GH-L127V*) was used to genotype Hereford (115 heads), Limousine (114 heads), Black-and-White (200 heads), Bestuzhev (200 heads) bull calves being fattened. The conducted research showed that there is a similar distribution of genotypes among bull calves of meat breeds with *GH^{LL}* homozygous genotype being more common (47.83% and 52.63%). Black-and-White and Bestuzhev bull calves have a higher frequency of *GH^{LV}* heterozygous genotype (62.50% and 59.0%). Hereford, Limousine and black-and-white bulls have a greater frequency of *GH^L* allele (0.69; 0.71; 0.51), Bestuzhev animals have a higher rate of *GH^V* allele (0.62). The paper presents the influence of somatotropin hormone gene polymorphism on some meat productivity and growth rate indicators of young animals. Thus, *GH^{LL}*-genotyped Hereford, Limousine, and Black-and-White bull calves had significantly higher live weight (pre-slaughter live weight) as well as absolute and average daily live weight gains at the end of rearing. According to the results *GH^{LV}*-genotyped animals were in the second place and *GH^{VV}*-genotyped bulls ranked the last. As a result of the one-way analysis of variance, there has been found a high proportion of the studied polymorphism factor in developing meat productivity and growth rate indicators for Limousine, Black-and-White and Hereford bull calves. Genotyping by the *GH* gene as an additional criterion can be used in the selection of animals to improve cattle meat quality.

KEY WORDS

Bestuzhev breeds, Black-and-White, GH, growth hormone gene, growth rate, Hereford, Limousine, PCR-RFLP, polymorphism.

INTRODUCTION

Beef production is still an important area to develop the meat industry in animal breeding. In spite of the fact that the number of meat cattle is rising due to domestic and im-

ported specialized breeds, about 90% of beef comes from cattle of dairy and combined breeds. Live weight is an important commercial indicator. It depends on many factors, including growth hormone. Domestic and foreign researchers claim that somatotropin, as a pancreatic hormone,

stimulates the transport of amino acids into cells, takes part in the exchange of proteins, carbohydrates, fats and thus, is one of the major regulators of somatic growth of the body (Curi *et al.* 2006; Lee *et al.* 2013; Kalashnikova *et al.* 2015; Pozovnikova *et al.* 2015; Lysenko *et al.* 2016; Beyshova *et al.* 2017). Genotyping of animals by the gene of somatotropin hormone (GH) has a certain scientific and practical significance, as it allows to establish the genetic potential of meat productivity of animals by the following indicators: live weight dynamics and gain, carcass weight, meat yield, and marbling. Growth hormone has a broad spectrum of action. Its allelic variants can have different significance for different indicators of meat productivity (Uryadnikov *et al.* 2011; Gorlov *et al.* 2014). The synthesis of GH growth hormone is controlled by a gene having four allelic variants: C, D, L, V (Kalashnikova *et al.* 2015). GH^C-to-GH^G nucleotide replacement in the 5th exon of the GH gene, leading to Leu- to-Val amino acid replacement, is associated with live weight gain and intramuscular fat deposition GH^{GG} (GH^{VV}) genotype in cattle is associated with marbling (Tatsuda *et al.* 2008). The results of many studies prove that the bull calves, homozygous for the first allele GH^{CC} (GH^{LL}), have higher live weight and lower marbling levels than those of GH^{CG} (GH^{LV}) and GH^{GG} (GH^{VV}) genotypes (Tatsuda *et al.* 2008; Soloshenko *et al.* 2011; Uriadnikov *et al.* 2011). Because the production of competitive beef is one of the most important issues of animal breeding, animal genotyping by the growth hormone gene becomes particularly relevant in cattle selection and breeding.

The aim of our research is to assess bull calves in terms of their genetic growth intensity potential. The investigation was conducted on four generation breeds bred in South Ural and being different in environmental and genetic characteristics due to their growth hormone gene polymorphism. There are following tasks to be solved: to determine the GH gene polymorphism of Hereford, Limousine, black-and-white and Bestuzhev bull calves; to study the effect of the gene polymorphism on the growth rate of young cattle.

MATERIALS AND METHODS

Animals

For genotyping by the growth hormone gene Hereford, Limousine, black-and-white and Bestuzhev bull calves were selected. These were one-month-aged animals bred in agricultural enterprises located in the southern Urals. There were 115 Hereford bull calves, the offspring (the second and third Russian generation) of the animals brought to the LLC SAVA-Argo-Usen from Australia, in particular from South Australia, Victoria, New South Wales states and the island of Tasmania in 2009. 114 Limousine calves, the offspring (fourth and fifth generation) of animals, bred at the LLC "SAVA-Agro-Yapryk" by accumulation cross-

breeding of Simmental cattle with servicing bulls of French selection. Both farms are located in the Tuimazy district of the Republic of Bashkortostan (the Cis-Ural steppe zone).

They use a drylot and pasture beef cow-calf production system with resource-saving elements (Gizatullin, Sedykh, 2016; Sedykh *et al.* 2017). Both farms are breeding farms for the corresponding breeds of specialized beef cattle. They keep a fattening stock to produce beef. Black-and-white and Bestuzhev bull calves (200 heads of each breed) have been studied in the agricultural production cooperative named after Lenin located in the Diurtiuli district of the Bashkortostan Republic. This enterprise is a breeding farm for Bestuzhev breed. Bull calves are fattened indoors on a deep litter by the free-stall housing technology.

Depending on the specified genotypes, bull calves of different GH gene genotypes were divided into groups by the live weight analogue method. Homozygous for the first allele - I group (n=20); heterozygous - II group (n=20); homozygous for the second allele - III group (n=10).

Beef cattle (Hereford and Limousine breeds) were grown and fattened according to the beef animal breeding technology under the same conditions until animals reached required slaughter characteristics. Black-and-White as well as Bestuzhev bull calves were also kept according to a certain technology, under the identical conditions, until the required slaughter characteristics.

Study of growth intensity

In the study of meat productivity, the growth rate of young Hereford, Limousine, black-and-white and Bestuzhev cattle their live weight at birth, absolute, average daily gain, the relative growth rate for the entire rearing period as well as pre-slaughter live weight (live weight at the end of fattening) were examined. The weighing was carried out on platform floor scales. The absolute increase in the body weight unit for the entire period of rearing was calculated by the formula (1).

$$A = W_t - W_0 \quad (1)$$

Where:

A: absolute live weight gain, (g).

W₀: initial weight of the animal, (kg).

W_t: live weight of the animal at the end of the period.

The average daily live weight gain for the entire rearing period was calculated by the formula (2):

$$C = (W_t - W_0) / t \quad (2)$$

Where:

C: average daily live weight gain, (g).

W₀: initial weight of the animal, (kg).

W_t: live weight of the animal at the end of the period.
t: rearing period (days).

The relative growth rate was indicated by the S.Brody formula (3):

$$K = (W_1 - W_0) / (0.5 \times (W_1 + W_0)) \times 100 \quad (3)$$

DNA extraction and genotyping

The genotyping was carried out in the laboratory of DNA technologies of the All-Russian Research Institute of Breeding and in the laboratory of molecular genetics of the Bashkir State Agrarian University. DNA isolation was performed by a standard phenol-chloroform method and using the Extran commercial DNA kit (Syntol). The *GH* gene polymorphism was detected by polymerase chain reaction restriction fragment length polymorphism (PCR-RFLP) (PCR-RFLP) with primers: F: 5'-TAG-GGG-AGG-GTG-GAA-AAT-GGA-3' R: 5'-GAC-ACC-TAC-TCA-GAC-AAT-GCG-3' (Kalashnikova *et al.* 2015). During PCR (30 cycles) the annealing temperature was 58 °C. The resulting gene amplicons were split by the AluI endonuclease.

The number and length of restriction fragments were determined electrophoretically in 7.5% PAGE in the UV light after staining with ethidium bromide. To analyse the visualization of gels the Gel Doc XR gel documentation system and the attached software Image Lab 2.0 "DNA-analyser" were used. The sizes of restriction fragments: GH^{LL} - 185,132,51,36 NS; GH^{LV} - 236,185,132,51,36 NS; GH^{VV} - 236,132,36 NS.

Statistical analysis

The expected heterozygosity (H_e) rate was calculated based on the data of the bull calves genotype analysis by DNA markers according to the formula (4):

$$H_e = 1 - \sum p_i^2 \quad (4)$$

Where:

pi: i-allele frequency.

The observed degree of heterozygosity (H_o) was calculated by the formula (5):

$$H_o = n / N \quad (5)$$

Where:

n: number of animals heterozygous for this allele.

N: sample size.

To assess the compliance of the actual and expected genotype distribution in the studied animal samples, χ^2 was used, calculated by the formula (6):

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

Where:

O and E: observed and theoretically expected amounts of genotypes of a certain type.

k: number of genotypic classes.

Statistical processing of the results of weighing young animals was carried out by standard methods using the software application "Excel" of "Microsoft Office".

There are reliable differences in growth intensity between GH^{LL} and GH^{VV} genotype animals. That's why a one-way variance analysis was performed to find the influence of the SNP GH-L127V factor on the studied indicators.

RESULTS AND DISCUSSION

The results of the study of *GH* gene polymorphism in animals of the studied breeds are given in Table 1.

Genotype distribution by the *GH* gene among the studied Hereford and Limousine animals has been found in the same. The homozygous GH^{LL} genotype is more common among bull calves of both beef breeds, with the heterozygous GH^{LV} genotype being the second and the GH^{VV} genotype being the third by frequency.

On the other hand, there are some differences between the studied groups of animals of the studied beef breeds. The tables 1 show a small predominance of the Hereford bull calves of GH^{LL} genotype (47.83%) over the heterozygous GH^{LV} genotype (41.74%) by 6.09%. There is a significant predominance of the GH^{LL} genotype (52.63%) over the GH^{LV} genotype in Limousine animals, with a difference in the frequency between these genotypes being 16.67%. The proportion of animals with the GH^{VV} genotype in the samples of both breeds does not differ significantly. The GH^L allele frequency is slightly higher in Limousine bulls (0.71), it was 0.69 in Hereford young stock.

Dairy and dual-purpose animals have the highest concentration of the heterozygous GH^{LV} genotypes (62.5% and 59.0% respectively in the samples of Black-and-white and Bestuzhev breeds). At the same time, there is low frequency of the genotypes, homozygous for the first allele among the young dairy cattle. Thus, there was 19.5% frequency of GH^{LL} genotype in the sample of the Black-and-White breed, 8.5% frequency in Bestuzhev animals being the lowest among the studied cattle.

Table 1 GH gene polymorphism in bull calves of different breeds

Breed	n	The genotype frequency						Frequencies of alleles	
		GH ^{LL}		GH ^{LV}		GH ^{VV}		GH ^L	GH ^V
		Heads	%	Heads	%	Heads	%		
Hereford	115	55	47.83	48	41.74	12	10.43	0.69	0.31
Limousine	114	60	52.63	41	35.96	13	11.40	0.71	0.29
Black-and-White	200	39	19.5	125	62.5	36	18.0	0.51	0.49
Bestuzhev	200	17	8.5	118	59.0	65	32.5	0.38	0.62

The highest frequency of GH^{VV} genotype was observed in the Bestuzhev breed being 32.5% and 18, 0% in the Black-and-White breed.

Thus, the Bestuzhev breed is significantly different from other studied breeds in allele frequencies, namely: compared to other studied samples, it has a twice higher frequency of GH^V allele and twice lower frequency of GH^L allele respectively.

Genetic variation can be observed when there are heterozygotes in the population. The results of actual and expected heterozygosity for the growth hormone gene are presented in Table 2.

The data in Table 2 proves that the heterozygosity of beef animals is slightly higher. It is more by 0.015 (compared to the Hereford breed) and 0.054 (compared to the Limousine cattle).

Pearson criterion values were not high. They indicate that both the studied populations of beef cattle are in relational balance.

In both studied samples of dairy breeds, there is a deviation from HW equilibrium with respect to the distribution of the genotype frequencies: there are excess heterozygotes in the black-and-white breed (62.6% vs. 50% expected) and their lack in the Bestuzhev breed (59% vs. 85.6% expected).

Live weight Indicators and live weight gain of bull calves of the studied breeds are given in Table 3.

In all studied breeds, GH gene polymorphism has no effect on the weight of calves at birth, but in the process of growth and development, there are certain regularities of its influence on the growth rate.

Hereford bull calves of GH^{LL} and GH^{VV} genotypes have certain differences in the studied indicators ($P < 0.05$). GH^{LL} genotype bulls have a larger live weight at the end of fattening, higher absolute and average daily gains. Thus, the difference between the above mentioned genotypes in terms of pre-slaughter live weight was 4.2%, in absolute live weight gain – 4.6% and the average daily live weight gain – 4.5%.

Indicators of pre-slaughter live weight, absolute and average daily gains of GH^{LL} genotyped Limousine bulls are significantly higher ($P < 0.01$) than those of GH^{VV} genotyped bulls by 4.8%; 5.0% and 5.0%, respectively.

There is also a significant difference ($P < 0.05$) between the groups of young animals GH^{LL} and GH^{LV}-genotyped in terms of average daily live weight gain (1.7%).

A similar trend is observed in the case of Black-and-White bulls. They, as well as specialized beef breeds, have significant differences between the GH^{LL} and GH^{VV} genotypes in pre-slaughter live weight, absolute and average daily gain with GH^{LL} genotyped animals being predominant by these indicators. Compared with GH^{VV} genotype bulls their pre-slaughter live weight was higher by 29.6 kg (7, 3%), the absolute increase was more by 30.7 kg (8.2 per cent). Accordingly, the average daily gain was also higher by 56.1 g (8.2%).

Bestuzhev bull calves of different genotypes by the GH gene have no significant differences in meat productivity. There are only a tendency of some advantage (by 1.5%) of GH^{LL} genotyped animals on pre-slaughter live weight, absolute and average daily gains for the whole rearing period.

Indicators of the relative growth rate witness that young Hereford and Limousine breeds grow more intensively than dual-purpose (Bestuzhev) and dairy (Black-and-White) cattle. It may be noted that young Bestuzhev and Black-and-White cattle have a higher relative growth rate among heterozygous and GH^{LL} genotyped bull calves. Among Limousine bull calves GH^{LL} genotyped animals have a higher growth rate. There is a tendency of higher relative growth rate in Bestuzhev cattle in the direction GH^{LL}→GH^{LV}→GH^{VV} genotypes.

A one-way analysis of variance of the SNP GH-L127V polymorphism influence on the indicators of pre-slaughter live weight, absolute and average daily gain by Hereford, Limousine and Black-and-white bull calves is given in Table 4.

The results of one-way analysis of variance indicate that the highest rate of the factor influence of the studied polymorphism of the somatotropin hormone gene was found in relation to the pre-slaughter live weight being 0.19 in Hereford cattle and 0.39 in Limousine animals.

There have been found different influence rates of the SNP GH-L127V polymorphism on the absolute and average daily live weight gain being 0.16 in the Hereford cattle and 0.38 in the Limousine stock.

Table 2 Actual and expected heterozygosity for the *GH* gene

Breed	H _o	H _e	F	χ ²
Hereford	0.417	0.432	-0.015	0.052
Limousinee	0.360	0.414	-0.054	0.704
Black-and-white	0.626	0.500	0.126	3.175
Bestuzhev	0.590	0.856	-0.266	8.266

H_o: observed heterozygosity; H_e: expected heterozygosity; F: H_o-H_e difference; χ²: pearson criterion, compliance of observed and expected genotype distribution.

Table 3 Live weight dynamics and gain for bull calves of different genotypes by *GH* gene

Indicator	Genotype		
	<i>GH</i> ^{LL} (n=20)	<i>GH</i> ^{LV} (n=20)	<i>GH</i> ^{VV} (n=10)
Hereford breed			
Live weight at birth, kg	33.4±0.2	32.9±0.4	33.5±0.4
Pre-slaughter live weight (at the end of fattening), kg	582.6±6.2	569.4±3.7	558.2±5.3*
Absolute increase in live weight, kg	549.8±6.6	537.8±3.7	524.7±5.3*
Average daily live weight gain, g	904.3±10.4	884.5±6.0	863.0±8.6*
Relative growth rate	178.5±12.1	178.6±10.8	177.3±13.0
Limousine breed			
Live weight at birth, kg	34.4±0.3	33.6±0.3	32.2±0.2
Pre-slaughter live weight (at the end of fattening), kg	608.2±2.7	598.7±3.2	579.0±5.2**
Absolute increase in live weight, kg	574.6±3.3	563.5±6.6	545.8±5.1**
Average daily live weight gain, g	945.1±4.8	929.4±5.2*	897.7±8.4**
Relative growth rate	178.8±15.0	178.2±13.2	178.6±10.7
Black-and-White breed			
Live weight at birth, kg	30.2±0.3	29.3±0.2	31.3±0.3
Pre-slaughter live weight (at the end of fattening), kg	433.6±5.2	422.4±2.8	404.0±5.3**
Absolute increase in live weight, kg	403.4±2.3	393.1±2.9	372.7±4.2**
Average daily live weight gain, g	737.2±5.7	718.4±6.2	681.1±5.1**
Relative growth rate	173.9±5.2	174.1±6.3	171.2±6.8
Bestuzhev breed			
Live weight at birth, kg	29.4±0.7	28.8±0.7	28.6±0.6
Pre-slaughter live weight (at the end of fattening), kg	452.3±7.2	449.0±5.1	445.3±8.8
Absolute increase in live weight, kg	422.9±4.5	420.2±3.2	416.7±3.1
Average daily live weight gain, g	772.8±6.2	767.9±7.3	761.5±5.5
Relative growth rate	175.6±5.6	175.9±5.5	175.9±4.6

** (P<0.01) and * (P<0.05).

Black-and-White bull calves had average influence rate of the studied polymorphism on indicators of live weight at the end of rearing (0.30), meanwhile influence rate on absolute and average daily gains was a little higher being 0.31.

In general, it should be noted that we have obtained high rates of the SNP *GH*-L127V polymorphism effect on the pre-slaughter live weight, absolute and average daily gains. This fact can be explained by the significant role of growth hormone in the process of somatic growth of the body. The greatest dependence of the studied indicators of meat productivity on the above mentioned polymorphism is observed in bulls of the Limousine and Black-and-white breeds.

When genotyping beef bulls, it was found that the somatotropin hormone gene genotype distribution is similar. The most common genotype is the homozygous *GH*^{LL} genotype (47.83% and 52.63%).

GH^L allele is more frequent in Hereford, Limousine and Black-and-white bull calves (0.69; 0.71; 0.51), *GH*^V allele is more common in Bestuzhev young bulls being 0/62.

The results of genotyping Hereford, Limousine, and Black-and-white cattle are consistent with the results of [Khatami et al. \(2005\)](#), [Gorlov et al. \(2014\)](#), [Sharipov et al. \(2014\)](#), [Pozovnikova et al. \(2015\)](#) and [Beyshova et al. \(2017\)](#).

Also, *GH*^{LL} genotyped bulls of the above-mentioned breeds had significantly higher live weight at the end of fattening (pre-slaughter live weight), and, accordingly, absolute and average daily gains. According to the studied indicators *GH*^{LV} genotyped animals ranked the second, *GH*^{VV} genotyped were the last.

The results received prove physiologically normal state of young Hereford, Limousine, Black-and-White and Bestuzhev cattle.

Table 4 Influence of SNP GH-L127V on some meat productivity indicators of bull calves of different breeds

Age, months	Cx	Cz	Cy	η^2	F-test	P-value
Hereford breed						
Pre-slaughter live weight, kg	4279.56	22335.27	26614.82	0.19	4.50	0.016
Absolute live weight gain during rearing, kg	4234.69	22085.3	26319.99	0.16	4.51	0.016
Average daily live weight gain during rearing, g	11456.19	59742.98	71199.18	0.16	4.51	0.016
Limousine breed						
Pre-slaughter live weight, kg	5831.32	9131.28	14962.60	0.39	15.00	9.118
Absolute increase in live weight, kg	5584.81	8958.43	14543.24	0.38	14.65	1.13
Average daily live weight gain, g	15107.37	24232.80	39340.18	0.38	14.65	1.13
Black-and-White breed						
Pre-slaughter live weight, kg	5862.40	13867.60	19730.00	0.30	9.93	0.001
Absolute increase in live weight, kg	6242.40	13867.60	20110.00	0.31	10.58	0.001
Average daily live weight gain, g	20941.26	46358.78	67300.04	0.31	10.61	0.001

Cx: between-group variance; Cz: intragroup variance; Cy: total variance and η^2 : influence power factor.

The established effect of the somatotropin hormone gene polymorphism on some indicators of the growth rate of young cattle of different breeds is consistent with the results of studies of Chrenek *et al.* (1998), Unanian *et al.* (1998), Grochowska *et al.* (2001), Tambasco *et al.* (2003), Pal *et al.* (2004), Curi *et al.* (2006), Tatsuda *et al.* (2008) and Sharipov *et al.* (2014). There is another vision in studies of Biswas *et al.* (2003), Oprzadek *et al.* (2005) and Pereira *et al.* (2005), where heterozygous GH^{LV} genotyped animals had higher live weight or there was reliable excess of average daily live weight gain among GH^{GG} genotyped bull calves (Lee *et al.* 2013).

As a result of the one-way analysis of variance, there has been found a fairly high rate of the SNP $GH-L127V$ gene polymorphism in developing the studied indicators of meat productivity in Limousine, Black-and-White and Hereford bull calves. The effect of polymorphism on the pre-slaughter live weight was 0.19 for Hereford bulls, 0.39 for Limousine bulls and 0.31 for Black-and-White bulls while absolute and average daily live weight gains were 0.16, 0.38 and 0.31 respectively.

CONCLUSION

The studies of beef calves showed a similar distribution of genotypes with GH^{LL} homozygous genotype of SNP GH-L-127V being dominant. While black-and-white and Bestuzhev cattle are mostly of heterozygous genotype. The polymorphism influence on the indicators of live weight, absolute and average daily weight gains have been found. Hereford, Limousine as well as Black-and-White bull calves are greatly dependent on a genotype. When conducting cattle selection and breeding in order to increase meat productivity breeding and commercial farms are recommended to consider genotyping by GH gene as an additional criterion to select animals.

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