

The effect of SNP polymorphisms in growth hormone gene on weight and linear growth in crossbred Red Angus × Kalmyk heifers

Foat Kayumov

*Department of beef cattle
breeding*

*Federal Research Centre of
Biological Systems and Agro-
technologies of the Russian
Academy of Sciences
Orenburg, Russia
nazkalms@mail.ru*

Vladimir Kosilov

*Faculty of Biotechnology and
Environmental Management*

*Orenburg State Agrarian
University
Orenburg, Russia
kosilov_vi@bk.ru*

Nikolay Gerasimov

*Department of beef cattle
breeding*

*Federal Research Centre of
Biological Systems and Agro-
technologies of the Russian
Academy of Sciences
Orenburg, Russia
nick.gerasimov@rambler.ru*

Olga Bykova

*Department of technology of
production and processing of
agricultural products*

*Ural State Agrarian University
Ekaterinburg, Russia
olbyk75@mail.ru*

Abstract— The aim of our research was to study the effect of single-nucleotide polymorphisms in growth hormone gene on variability of weight and linear growth in crossbred Red Angus × Kalmyk heifers (F₂). The experimental animals were reared in “Agrofirma Aduchi” Ltd. in Tselinniy region, Republic of Kalmykia. Heifers were divided into groups after genotyping in accordance with the allelic variant of the growth hormone gene: I group completed with GH^{LV} heterozygous animals (n = 11 heads), II group – representatives of the homozygous GH^{VV} gene (n = 9 heads). The inter-group differences in live weight were insignificant (P > 0.05) between the carriers of various genotype variants in growth hormone gene in the weaning age (8 months). The advantage in live weight between experimental animals increased to 23.9 kg (P < 0.05) at the final stage of the control rearing (18 months). In this period, heterozygous youngsters (GH^{LV}) differed in a large format of the exterior compared to homozygous contemporaries (GH^{VV}). The significant superiority was established absolutely for all studied measurements. The maximum effect of heredity factor on the variability of live weight was detected at the age of 18 months – 24.81% (P < 0.05). The calculation of the organized factor impact to the overall variability of linear growth in heifers of different allelic variants for the GH gene showed significance (P < 0.05) at 18 months of age. Thus, the detection of single-nucleotide polymorphisms in GH gene can improve the accuracy of the selection in beef herds.

Keywords— *heifers, live weight, linear growth, genotype, allele, growth hormone gene.*

I. INTRODUCTION

The development of agricultural production received a significant impulse after the most important discoveries in the field of genetics and molecular biology. So, recently, the use of single-nucleotide substitutions in the gene sequence had obtained the applied course [1]. Genotyping of animals and identification of carriers of “desirable” alleles in different genes showed a number of reliable associations with quantitative and qualitative traits [2-4]. In particular, polymorphisms in genes determining the nature of adipose metabolism (Lep, RORC, DGAT1, TG5, etc.) in cattle reliably connected with the marbling of beef and backfat depth [5-8]. Functional substitutions in the nucleotide sequence were also revealed in the genes of calpain

(CAPN1) and calpastatin (CAST), which cause the formation of meat tenderness [9-11]. In addition, a positive association of single nucleotide polymorphisms in GDF5 gene with the development of bone tissue, ligaments and tendons was found. The selection of carriers taking into account the “desirable” allelic variant in this gene will contribute to the formation of large-framed and tall animals, which meets modern requirements for high-tech beef cattle [12]. There are several polymorphisms in somatotropin gene (GH). The bovine DNA fragment encoding the growth hormone is localized on the 19th chromosome and consists of 5 exons and 4 introns (BTA 19, NCBI Reference Sequence AC_000176.1). The AluI polymorphism is associated with the replacement of bases C-G in the 5th exon of the bGH gene, as a result the amino acid Leu is replaced with the amino acid Val (L127V, rs 4192384). There is an association of various polymorphic variants of the bGH gene with different productive traits in cattle [13].

The aim of our research was to study the effect of single-nucleotide polymorphisms in growth hormone gene on variability of weight and linear growth in crossbred Red Angus × Kalmyk heifers (F₂).

II. MATERIALS AND METHODS

The object of the study were crossbred Red Angus × Kalmyk heifers (F₂). The experimental animals were reared in “Agrofirma Aduchi” Ltd. in Tselinniy region, Republic of Kalmykia. The management system was the same for heifers of all groups and organized in accordance with the traditional technology adopted in beef cattle breeding.

Blood samples were taken from jugular vein of experimental animals to determine single nucleotide polymorphisms in GH genes (somatotropin). Blood was injected into test tubes with 600 μl ethylenediaminetetraacetic acid (EDTA) to obtain a volume of 10 ml. Genomic DNA was extracted from whole blood using the reagent kit “DIAtom™ DNA Prep” (IsoGene Lab, Moscow) in laboratory of immunogenic and DNA-technologies of All-Russian Research Institute of sheep and goat breeding – branch of the North Caucasus federal agricultural Research Center. A “GenePak™ PCR Core” kit

(IsoGene Lab, Moscow) were used to conduct the polymerase chain reaction.

Genotyping was performed using PCR-RFLP using a "Tertsik" programmable thermal cycler (DNA technology, Russia) to assess the polymorphisms in somatotropin (GH) gene. The amplification of the sites was conducted using primer with following sequence: F: 5'-gct-gct-cct-gag-ggc-cct-tcg-3'; R: 5'-gcg-gcg-gca-ctt-cat-gac-cct-3'.

The size of the amplified fragment was 223 p.n. PCR program for the GH gene: "hot start" - 5 min. at 95°C; 35 cycles: denaturation - 45 seconds at 94°C, annealing - 45 seconds at 65°C, synthesis - 45 seconds at 72°C; completion - 7 minutes at 72°C. The endonuclease *AluI* was used for restriction of the amplified regions of the genes. The splitting of the products was carried out at 37°C, the genotypes were identified by gel electrophoresis with visualization under UV light. Product identification for somatotropin gene: GH^{VV} - 223 p.n.; GH^{LV} - 223, 171, 52 p.n.; GH^{LL} - 171, 52 p.n.

Heifers were divided into groups after genotyping in accordance with the allelic variant of the growth hormone gene: I group completed with GH^{LV} heterozygous animals (n = 11 heads), II group - representatives of the homozygous GH^{VV} gene (n = 9 heads).

Weight growth of heifers was studied by monthly weighing of animals in the morning before feeding. Linear growth was determined by taking basic body measurements (withers height, hip height, body length, chest width, chest depth, chest girth, hip width, metacarpus girth) at the age of 8 and 18 months.

Statistical analysis. The genetic characteristics of cattle by GH gene including genotypic and allelic frequencies, observed heterozygosity (H_o) and expected heterozygosity (H_e), effective allele numbers (N_e), Hardy-Weinberg equilibriums were calculated according to Nei (1978).

Frequencies of different genotypes were estimated according to the following statistical model:

$$p = n/N,$$

where p - genotypic frequencies, n - number of individuals with a specific genotype, N - total number of individuals.

Frequencies of different alleles were estimated according to the following statistical equation:

$$P_A = (2nAA + nAB) \div 2N,$$

$$q_B = (2nBB + nAB) \div 2N,$$

where P_A - allelic "A" frequencies, q_B - allelic "B" frequencies, N - total number of alleles.

The expected genotypic frequencies in the studied population were calculated according to the Hardy-Weinberg equilibrium.

The effective number of alleles was estimated according to the following statistical equation:

$$N_s = \frac{1}{1 - H_s}$$

where H_e - expected heterozygosity

The reliability level of the obtained results was determined by the criteria χ^2 .

Data were processed with one-way analysis of variance with using Statistica 10.0 software Generalized Linear Models procedures (Statsoft Inc., 2009). Least squares differences and probability values for differences were calculated using Tukey's HSD for unequal N.

III. RESULTS

The creation of effective type of beef cattle is based on the genetic control of the herd by the frequency of the desirable alleles in DNA markers associated with performance and meat productivity. Thus, testing of crossbred heifers by single nucleotide polymorphisms of the growth hormone (GH L127V) gene indicated different genetic frequency (Table 1). There were no carriers of the "desirable" VV genotype when analyzing the frequency distribution in GH gene locus, while the homozygous GH^{LL} variant was found in 63% of heifers. The study of the allelic ratio in the locus showed a significant distribution of L allele in the herd, whose frequency was 0.64 units higher than the "desirable" allele, which is associated with growth intensity in cattle.

TABLE I. GENETIC CHARACTERISTICS OF CROSSBRED RED ANGUS × KALMYK (F2) HEIFERS (N=30 ANIMALS)

Gene	Genotypic frequency			Allelic frequency		N _e ^a	H _e ^b	χ ²
	VV	LV	LL	V	L			
bGH	0	0.37	0.63	0.18	0.82	1.419	0.295	1.549

^a. Effective number of alleles
^b. Expected heterozygosity

The expected heterozygosity index (H_e) in the studied DNA marker of meat productivity had some deviation from the equilibrium distribution of genotype frequencies at the GH locus, as evidenced by the ratio of observed genotypes and theoretically expected in accordance with Hardy-Weinberg equation. However, the gene imbalance ($\chi^2 = 1.549$) did not reach a significant level in the heifer group.

The differences in live weight between carriers of different genotype variants for growth hormone gene were insignificant in the period from birth to weaning (Table II). Thus, the maximum birth weight was observed among heterozygous (GH^{LV}) heifers, the superiority was 1.2 kg (4.94%; P> 0.05). By 8 months of age, this difference reached 2.3 kg (1.10%; P> 0.05).

TABLE II. THE DYNAMICS OF LIVE WEIGHT IN CROSSBRED RED ANGUS × KALMYK (F2) HEIFERS DEPENDING ON THE GH L127V GENOTYPE (M±SE)

Age, months	Genotype	
	GH ^{LV}	GH ^{VV}
At birth	25,5±1,21	24,3±0,50
8	211,2±4,18	208,9±5,06
12	303,9±6,07 ^a	283,1±4,37
15	367,2±7,40 ^a	345,8±4,51
18	409,5±7,27 ^a	385,6±6,26

^a The factor has a statistically significant effect, P<0.05

Probably, the low milk productivity of dams hindered the full realization of the growth potential in heifers, which had the desirable allele (L) in somatotropin gene. After weaning, heterozygous individuals showed the genetic capacity of intensive development. Thus, at the age of 12 months, the superiority of GH^{LV} genotype carriers increased to 20.8 kg (7.35%; P<0.05). Further advantage in size of live weight

between experimental animals increased, and reached the maximum level of 23,9 kg (6,20; P<0,05) to 18-month age.

Probably, the low milk productivity of dams hindered the full realization of the growth potential in heifers, which had the desirable allele (L) in somatotropin gene. After weaning, heterozygous individuals showed the genetic capacity of intensive development. Thus, at the age of 12 months, the superiority of GH^{LV} genotype carriers increased to 20.8 kg (7.35%; P<0.05). Further advantage in size of live weight between experimental animals increased, and reached the maximum level of 23,9 kg (6,20; P<0,05) to 18-month age.

Thus, a full realization of the genetic potential of weight growth was observed in heifers-carriers of the desired allele in growth hormone gene locus at the late stages of ontogenesis, which indicates a long maturity of heterozygous individuals. This can be judged by changes in the average daily gain in different age periods of control rearing (table. III). Thus, the growth rate of experimental heifers was at the same level at the suckling stage of rearing (from birth to 8 months): 759.5-763.9 g per day. After weaning, the heterozygous individuals for the GH^{LV} gene differed in the best weight gain, which exceeded their peers by 151.7 g (24.93; P <0.05). This advantage was provided by a sharp slowdown in growth rate of homogeneous genotype VV carriers during puberty, which indicates their precocity.

TABLE III. AVERAGE DAILY GAIN IN CROSSBRED RED ANGUS × KALMYK HEIFERS (F₂) DEPENDING ON THE GH L127V GENOTYPE (M±SE)

Age period, months	Genotype	
	GH ^{LV}	GH ^{VV}
0-8	763,9±16,86	759,5±20,47
8-12	760,1±27,19 ^a	608,4±52,90
12-15	695,3±29,00	688,6±34,12
15-18	465,5±27,81	437,1±26,59
8-18	652,5±18,32	581,1±28,87
0-18	702,0±13,02 ^a	660,4±11,57

^a The factor has a statistically significant effect, P<0,05

The intergroup differences were not so pronounced in the next stages of the control rearing. However, there was still an advantage in the indicator value in carriers of heterozygous genotype. During the post-weaning period (8–18 months), the difference was 71.4 g (12.29%; P> 0.05). There was a significant superiority of carriers of the desirable allele (L) in the average daily gain of 41.6 g (6.30%; P <0.05) for the entire period of the experiment (from birth to 18 months).

There were no significant intergroup differences in the magnitude of linear measurements development at the age of 8 months (Table IV).

Meanwhile, heifers with homozygous VV genotype in growth hormone gene exceeded peers in withers height by 0.3 cm (0.29%), hip height - by 0.4 cm (0.37%), chest width – by 0.3 cm (1.05%), hip width – by 0.5 cm (1.63%). At the same time, individuals with an LV allelic composition had an advantage in chest depth by 1.3 cm (2.11%), body length - by 1.2 cm (1.10%), chest girth - by 0.8 cm (0.62%) and metacarpus girth - by 0.2 cm (1.34%).

TABLE IV. THE DYNAMICS OF BODY MEASUREMENTS (CM) IN HEIFERS AT THE AGE OF 8 MONTHS (M±SE)

Body measurement	Genotype	
	GH ^{LV}	GH ^{VV}
Withers height	104.4±1.27	104.7±1.14
Hip height	107.5±1.30	107.9±1.25
Body length	110.4±1.09	109.2±1.15

Chest width	28.5±0.74	28.8±1.01
Chest depth	48.5±0.58	47.2±0.76
Chest girth	129.5±1.06	128.7±1.30
Hip width	30.7±1.09	31.2±1.32
Metacarpus girth	15.1±0.21	14.9±0.26

The low milk productivity of dams restrained the full realization of linear growth potential in heifers, which had a desirable allele (L) at the locus of the GH gene. After weaning, heterozygous individuals showed genetic capacity of intensive development (Table V). Thus, the superiority of GH^{LV} genotype carriers in live weight increased to 23.9 kg (6.20; P<0.05) at the age of 18 months.

TABLE V. THE DYNAMICS OF BODY MEASUREMENTS (CM) IN HEIFERS AT THE AGE OF 18 MONTHS (M±SE)

Body measurement	Genotype	
	GH ^{LV}	GH ^{VV}
Withers height	123.5±1.21 ^a	119.6±0.99
Hip height	127.4±1.21 ^a	123.2±0.80
Body length	146.5±1.30 ^a	142.3±0.94
Chest width	38.5±0.80 ^a	36.0±0.75
Chest depth	60.9±0.86	58.4±0.67
Chest girth	167.5±1.52 ^a	163.0±0.82
Hip width	41.9±0.90	39.9±1.03
Metacarpus girth	18.5±0.25	17.9±0.20

^a The factor has a statistically significant effect, P<0.05

Heterozygous young animals are characterized by their large format of exterior compared to their homozygous peers. The significant superiority was established absolutely for all studied measurements. Thus, the carries of GH^{LV} genotype significantly (P<0.05) exceeded their analogues in withers height by 3.9 cm (3.26%), hip height - by 4.2 cm (3.41%), chest width - 2.5 cm (6.94%), body length - by 4.2 cm (2.95%) and chest girth - by 4.5 cm (2.76%).

Analysis of variance of the data suggests that the effect of the hereditary factor (allelic composition in GH gene) on the variability of live weight in heifers is significant (P <0.05) and amounts to 24.81% of the sum of all influencing factors at 18 months of age (Fig. 1).

Minimum determination by the genotype were differed the following measurements: hip width (10.86%; P>0.05) and metacarpus girth (14.14%; P>0.05). The calculation of the organized factor impact to the overall variability of linear growth in heifers of different allelic variants for the GH gene showed significance (P <0.05) at 18 months of age. Thus, the share of genotypic contribution to withers height development was 25.44%, hip height - 29.17%, chest depth - 21.00%, chest width - 22.46%, body length - 25.11%, chest girth - 25.25%.

It should be noted that during weaning period (8 months) the force of genotype influence on realization of weight and linear growth potential decreases sharply. Thus, the impact of the hereditary factor on the variability of live weight had reached 0.69% (P>0.05) by the end of suckling stage of rearing. The development of body measurements in animal were also minimally determined by the allelic state of the growth hormone gene (P> 0.05). The strong influence of milk productivity of dams on the growth of young animals had limited the narrow range of intergroup variability of the traits.

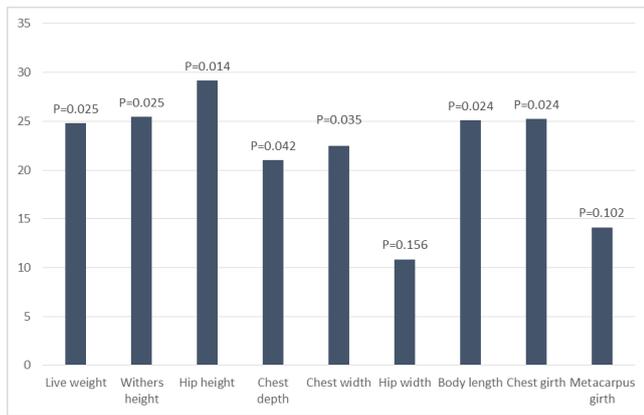


Fig. 1. The effect of genotype (%) in growth hormone gene on the variability of live weight and linear measurements in heifers at the age of 18 months

IV. CONCLUSION

When assessing the potential for weight and linear growth in crossbred Red Angus × Kalmyk (F2) heifers with regard to the genotyping for the growth hormone gene was found a significant effect of the genotype on the variability of live weight and body measurements at later stages of ontogenesis (after weaning from dams). The presence of allele L in the locus of GH gene is associated with the best development and formation of large-framed exterior in young animals. Thus, the detection of single-nucleotide polymorphisms in GH gene can improve the accuracy of the selection in beef herds.

REFERENCES

- [1] E.A. Rusakova, D.B. Kosyan, and S.A. Miroshnikov, "SIRT3, SIRT1 as potential regulation candidate genes of lipid metabolism in cattle (review)," *Animal Husbandry and Fodder Production*, vol. 101 (4), 2018, pp. 35-42.
- [2] A.L. Van Eenennaam, J. Li, R.M. Thallman, R.L. Quaas, M.E. Dikeman, C.A. Gill, D.E. Franke, and M.G. Thomas, "Validation of commercial DNA tests for quantitative beef quality traits," *J. Anim. Sci.*, vol. 85, 2009, pp. 891-900.
- [3] L.G. Jennifer, C.B. Stephen, and C. McCorquodale, "Association of selected SNP with carcass and taste panel assessed meat quality traits in a commercial population of Aberdeen Angus-sired of cattle," *Genet. Selection Evol.*, vol. 14, 2009, p. 36.
- [4] J.L. Gill, S.C. Bishop, C. McCorquodale, J.L. Williams, and P. Wiener, "Association of selected SNP with carcass and taste panel assessed meat quality traits in a commercial population of Aberdeen Angus-sired beef cattle," *Genet. Sel. Evol.*, vol. 41, 2009, (doi 10.1186/1297-9686-41-36).
- [5] T.W. Geary, E.L. McFadin, M.D. MacNeil, E.E. Grings, R.E. Short, R.N. Funston, and D.H. Keisler, "Leptin as a predictor of carcass composition in beef cattle," *J Anim Sci*, vol. 81(1), 2003, pp. 1-8.
- [6] E.L. McFadin, D.H. Keisler, T.B. Schmidt, C.L. Lorenzen, and E.P. Berg, "Correlations between serum concentrations of leptin and beef carcass composition and quality," *Journal of Muscle Foods*, vol. 14(1), 2003, pp. 81-87.
- [7] J.D. Nkrumah, C. Li, J. Yu, C. Hansen, D.H. Keisler, and S.S. Moore, "Polymorphisms in the bovine leptin promoter associated with serum leptin concentration, growth, feed intake, feeding behavior, and measures of carcass merit," *J Anim Sci*, vol. 83(1), 2005, pp. 20-28.
- [8] E. Casas, S.N. White, S.D. Shackelford, T.L. Wheeler, M. Koohmaraie, G.L. Bennett, and T. P. L. Smith, "Assessing the association of single nucleotide polymorphisms at the thyroglobulin gene with carcass traits in beef cattle," *J. Anim. Sci.*, vol. 85, 2007, pp. 2807-2814.
- [9] K. Kaplanova, A. Dufek, E. Drackova, J. Simeonova, and J. Subrt, "The association of CAPN1, CAST, SCD, and FASN polymorphisms with beef quality traits in commercial crossbred cattle in the Czech Republic," *Czech J. Anim. Sci.*, vol. 58, 2013, pp. 489-496.
- [10] E. Casas, S.N. White, T.L. Wheeler, S.D. Shackelford, M. Koohmaraie, D.G. Riley, C.C. Chase Jr., D.D. Johnson, and T.P.L. Smith, "Effects of calpastatin and μ -calpain markers in beef cattle on tenderness traits," *J. Anim. Sci.* vol. 84, 2006, pp. 520-525.
- [11] B.T. Page, E. Casas, M.P. Heaton, N.G. Cullen, D.L. Hyndman, C.A. Morris, A.M. Crawford, T.L. Wheeler, M. Koohmaraie, J.W. Keele, M.E. Dikeman, and T.P.L. Smith, "Evaluation of single-nucleotide polymorphisms in CAPN1 for association with meat tenderness in cattle," *J. Anim. Sci.*, vol. 80(12), 2002, pp. 3077-3085.
- [12] Y.F. Liu, L.S. Zan, K. Li, S.P. Zhao, Y.P. Xin, Q. Lin, W.Q. Tian, and Z.W. Wang, "A novel polymorphism of GDF5 gene and its association with body measurement traits in *Bos taurus* and *Bos indicus* breeds," *Mol. Biol. Rep.*, vol. 37(1), 2010, pp. 429-434 (doi: 10.1007/s11033-009-9604-5).
- [13] J.-H. Lee, Y.-M. Lee, J.-Y. Lee, D.-Y. Oh, D.-J. Jeong, and J.-J. Kim, "Identification of single nucleotide polymorphisms (SNPs) of the bovine growth hormone (bGH) gene associated with growth and carcass traits in Hanwoo," *Asian-Austral. J. Anim. Sci.*, vol. 26, no. 10, 2013, pp. 1359-1364.
- [14] M. Nei, "Estimation of average heterozygosity and genetic distance from a small number of individuals", *Genetics*, vol. 89 (3), 1978, 583-590.