Technology for detecting highly productive animals based on elemental status assessment

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Abstract. The studies were conducted on bull-calves of the Kalmyk breed (n = 100), age 12 months, live weight 326.4 ± 6.4 kg. In the course of the research, we performed a sampling of animal biosubstrates. The article studies a single nucleotide polymorphism of the growth hormone gene (rs135322669). The elemental composition of biosubstrates was determined by 25 indicators, by atomic emission and mass spectrometry (AES-ICP and MS-ICP). It was found that the frequency of occurrence of CC alleles in the sample was 62.0 %, CG – 26.0 %, and GG – 12.0 %, \( \chi^2 = 39.924, \chi^2 \text{ test} = 5.99 \). Evaluation of the elemental status of calf bull revealed significant differences depending on the polymorphism in the bovine growth hormone gene. In bulls with the CC genotype, the exchange pool Ca, K, Na, Co, Cr, Cu, J, Se, B, Si, Li, V was significantly higher compared to the CG genotype; and Ca, K, Na, J, Se, B, Li in comparison with the GG genotype. CC genotypes to GG were characterized by the accumulation of toxic elements: Al, Pb, Hg, Cd. Consequently, under the same ecological conditions of breeding in calf bull with the CC genotype, the detoxification mechanisms are higher, which is reflected in the reduced concentration of toxic elements in the wool. A technology for identifying highly productive animals is proposed based on the selection of bulls by the level of toxic elements in wool, calculated on the basis of the sum of moles: Al, Cd, Hg, Pb, Sn, Sr at 8 months of age, which allows increasing productivity by 9.4-15.6 %. It was concluded that there is a need to continue the research with the obligatory safety assessment of the toxic elements of the resulting meat products.

1. Introduction
The GH (growth hormone) gene is one of the genes closely related to the productive qualities of farm animals, including the ability to grow [1], reproductive qualities [2], milk production [3]. It acts as a candidate gene in the MAS program (Marker Assisted Selection) [4].

Due to the fact that GH directly or indirectly plays a significant role in tissue growth and regulation of fat metabolism [5, 6], we conducted a detailed study of mineral metabolism characteristics in animals with polymorphism for this gene. It becomes possible with a detailed study of the elemental status of animals by the wool composition as an integral indicator of exchange pools and the activity of chemical elements in the organs and tissues of the animal’s body. This is determined both by the close relationship between the concentration of trace elements in wool and blood of cattle [7,8], and the information content of cow wool as a long-term parameter for assessing the state of mineral metabolism [9, 10].
In this regard, an assessment of the elemental status of bull-calves depending on the polymorphism of bGH gene will give comprehensive information about its effect on the metabolism of mineral substances.

**Research goal.** The goal of the article is to study the effect of polymorphism in the bGH gene on the elemental status of Kalmyk breed bull-calves.

### 2. Material and research methods

The protocol of the present investigation was approved by the Local Ethics Committee of the Orenburg State University, Orenburg, Russia. All animal studies have been performed in accordance with the ethical standards of the 1964 Declaration of Helsinki and its later amendments.

**2.1. Experiment layout**

The study was conducted in 2020 at the SEC of the Krasnogorsk collective farm of the Orenburg region, Russia. At the first stage, blood samples were taken to identify one nucleotide polymorphism (SNP) (C / G, rs135322669) in Kalmyk breed bull-calves, age 12 months (n = 100). Blood samples were taken in the morning before feeding and drinking. Blood was taken from the tail vein from the middle third of the body of 2–5 tail vertebra and placed into vacuum tubes.

At the second stage of the research, wool samples were taken from the upper part of the withers in an amount of at least 0.4 g according to the proposed method [11]. The samples taken include 12 heads from each identified group of animals using a HeinigerSaphir wireless machine (Switzerland).

**2.2. Selection and testing of blood samples**

DNA samples were obtained from whole blood. The DNA isolation protocol was carried out in accordance with instructions of the commercial kit for isolation of genomic DNA from blood "DNA-Extran-1". The quality and quantity of nucleic acid was measured using a nanodrop ND-1000 spectrophotometer. The genomic DNA of each animal was stored at a temperature of -20 °C.

Primers were developed based on published bGH sequences (GenBank Accession NOS. M57764) using Primer3 software (www.genome.wi.mit.edu). The nucleotide sequence of the primer and the PCR conditions are shown in tables 1 and 2.

**Table 1. Specific oligonucleotides and program**

| Gene | SNP name | Location | Source | SNP | Sequence of primers |
|------|----------|----------|--------|-----|---------------------|
| GH   | GH-H1    | 47-558   | GenBank accession rs135322669 | G/C | (F)GGGGGTATGAGAAGCTG AAGGACCTG (R)CAGGAGCTGGAAAGATGG CACGACAC[12] |

Real-time PCR was performed on an ANK-32 programmer using 25 μl of the reaction mixture containing 60 μM Tris-HCl (pH 8.5), 1.5 μM MgCl2, 25 μM KCl, 10 μM mercaptoethanol; 0.1 μM Triton X-100; 0.2 μM dNTP, 1 unit Taq DNA polymerase, 0.5 μM of each of the primers. Amplification of the SNP GH-H1 gene was performed according to the regime indicated in table 2.

**Table 2. DNA amplification scheme**

| SNP name | Temperature, °C | Cycle |
|----------|-----------------|-------|
| GH-H1    | + 95 °C         | 120 c x 1 |
|          | + 63 °C         | 40 c x 40 |
|          | + 95 °C         | 20 c x 40 |

**2.3. Study of wool samples**

The elemental composition of wool was determined by atomic emission and mass spectrometry (AES-ICP and MS-ICP) in the INPO Test Center “Center for Biotic Medicine”, Moscow (Registration
Certificate of ISO 9001: 2000, Number 4017 – 5.04.06. Biosubstrateashing was carried out using the MD-2000 microwave decomposition system (USA). The content of elements in the resulting ash was estimated using an Elan 9000 mass spectrometer (Perkin Elmer, USA) and an Optima 2000 V atomic emission spectrometer (Perkin Elmer, USA). The elemental composition of biosubstrates was studied by 25 indicators (Al, As, B, Ca, Cd, Co, Cr, Cu, Fe, I, K, Li, Mg, Mn, Na, Ni, P, Pb, Se, Si, Sn, Hg, Sr, V, Zn).

2.4. Statistical processing
The study used the Shapiro – Wilk test to test the hypothesis of normal distribution of quantitative traits. The distribution law of the studied numerical indicators did not differ from the normal one. Therefore, the significance of the differences was checked using the generally accepted parametric method (Student t-test). We calculated the achieved significance level (p) in all statistical analysis procedures, while the critical significance level in this study was assumed to be less than or equal to 0.05. We used the Statistica 10.0 application package to process the data (Stat Soft Inc., USA).

3. Results
Detection of SNP (rs135322669) in the bGH gene showed three genotypes of the "C> G mutation". (picture 1).

![Figure 1](image1.png)

**Figure 1.** Frequency of occurrence of genotypes by marker bGH, heads

The frequency of occurrence of CC alleles in the sample is 62.0 %, CG – 26.0 and GG – 12.0 % ($\chi^2_{\text{cal}} = 39.924, \chi^2_{\text{test}} = 5.99 (P<0.05)$, with the frequency of alleles $C = 0.75; G = 0.25$).

The study of wool elemental composition from the withers of bull-calves of various genotypes made it possible to establish the following characteristics (Table 3).

A comparative assessment of chemical composition of Kalmyk breed bull-calves’ wool revealed a significant difference in element concentrations depending on the polymorphism in the bGH gene. So, the wool of animals with the CC genotype had more Ca, K, Na, Co, Cr, Cu, J, Se, B, Si, Li, V in comparison with the genotype CG; it also had more Ca, K, Na, J, Se, B, Li in comparison with the genotype GG. Polymorphism of the gene from CC to GG was accompanied by the accumulation of toxic elements: Al, Pb, Hg. Moreover, the differences in individual elements exceeded 100 % (Figure 2).

Hg was higher in the wool of bull-calves with the CG genotype, it accounted for 129.2 % ($P<0.001$), Al – in 113.1 % ($P<0.001$), Pb – in 57.4 % ($P<0.001$), with less Ca concentration – by 30.8 % ($P<0.001$), K – by 51.3 % ($P<0.001$), Na – by 45.9 % ($P<0.001$), Co – by 29.7 % ($P<0.05$), Cr – by 38.1 % ($P<0.05$), Cu – by 11.8 % ($P<0.05$), Fe – by 36.2 % ($P<0.01$), I – by 25.4 % ($P<0.001$),
Se – by 14.2% \( (P \leq 0.001) \), B – by 29.7% \( (P \leq 0.001) \), Si – by 51.4% \( (P \leq 0.05) \), Li – by 39.0% \( (P \leq 0.001) \), V – by 30.5% \( (P \leq 0.05) \) in comparison with CC.

**Table 3.** Concentration of chemical elements in wool of bull-calves of different genotypes, mg/kg (M ± STD)

| Element | Genotype | CC    | CG     | GG     |
|---------|----------|-------|--------|--------|
|         |          | 4356±627 | 3012±871\(^a\) | 3399±602\(^b\) |
|         |          | 6764±1145 | 3291±2136\(^a\) | 2650±882\(^b\) |
| Ca      |          | 813.1±244.4 | 602.9±354.3 | 695.6±139.1 |
| Mg      |          | 1846.08±272.02 | 998.92±408.80\(^a\) | 879.83±202.79\(^b\) |
| Na      |          | 242.17±55.83 | 241.58±52.64 | 279.92±53.74 |
|         |          | 4.19±0.46 | 3.95±0.69\(^a\) | 3.14±0.32\(^b\) |
| B       |          | 2.70±1.52 | 1.31±1.59\(^a\) | 2.55±1.02\(^c\) |
|         |          | 1.68±0.31 | 1.02±0.48\(^a\) | 1.04±0.25\(^b\) |
| Si      |          | 5.26±2.22 | 3.84±3.14 | 4.96±1.97 |
| Li      |          | 3.15±1.12 | 2.19±1.02\(^a\) | 2.53±0.68 |
|         |          | 0.21±0.030 | 0.17±0.092 | 0.34±0.079\(^c\) |
| As      |          | 233.8±86.2 | 498.3±65.4\(^a\) | 643.4±53.3\(^b\) |
| Sr      |          | 19.47±5.41 | 16.86±6.35 | 18.69±3.62 |
| Pb      |          | 0.235±0.081 | 0.370±0.040\(^a\) | 0.483±0.060\(^b\) |
| Sn      |          | 0.013±0.008 | 0.012±0.009 | 0.017±0.005 |
| Cd      |          | 0.03±0.01 | 0.02±0.01 | 0.04±0.01\(^c\) |
| Hg      |          | 0.012±0.007 | 0.028±0.005\(^a\) | 0.022±0.008\(^b\) |

\(^a\) – \( P \leq 0.05 \) – GG compared with CC;
\(^b\) – \( P \leq 0.05 \) – CC compared with GG;
\(^c\) – \( P \leq 0.05 \) – CC compared with CG.
The elemental composition of withers wool in animals with the genotypes of CC and GG had similar differences (Figure 3).

It was established that bulls with homozygous genotype G had an increased concentration of Al – by 175.2 % \((P \leq 0.001)\), Pb – by 105.7 % \((P \leq 0.001)\), Hg – by 80.6 % \((P \leq 0.01)\), As – by 61.4 % \((P \leq 0.001)\), Cu – by 11.7 % \((P \leq 0.05)\), with a reduced concentration of Se – by 15.0 % \((P \leq 0.001)\), Ca – by 22.0 % \((P \leq 0.001)\), B – by 25.0 % \((P \leq 0.001)\), I – by 25.4 % \((P \leq 0.001)\), Fe – by 31.1 % \((P \leq 0.001)\) Li – by 38.2 % \((P \leq 0.001)\), Na – by 52.3 % \((P \leq 0.001)\), K – by 60.8 % \((P \leq 0.001)\) in comparison with peers with genotype CC.

This prompted us to assess the relationship between the sum of macroelements (Ca, K, Mg, Na, P), essential (Zn, Fe, Cu, Mn, I, Se, Cr, Co), toxic (Al, As, Sr, Pb, Sn, Cd, Hg) elements expressed in mmol of substance with average daily gains of bull-calves. For this, Spearman's rank correlation analysis was carried out for all experiment bull-calves. As a result, the presence of reliable correlations between the average daily gain in live body weight and the amount of mmol of toxic trace elements \((r = -0.69)\); as for the amount of mmol of macroelements \((r = 0.34)\) and essential trace elements \((r = 0.32)\), no reliable relationship was found.
Taking into account the above, we proposed the use of the sum of toxic (Al, Cd, Hg, Pb, Sn, Sr) elements in the wool (mmol/kg) of the substance as a criterion for selecting bull-calves when forming productivity groups for feeding. For the calculation, it is proposed to use the formula:

\[ \sum_{\text{tox}} = \text{Al} + \text{Cd} + \text{Hg} + \text{Pb} + \text{Sn} + \text{Sr}, \]

where \( \sum_{\text{tox}} \) is the total toxic load of the body, mmol/kg.

The groups were formed by the centile method: I group – <25 percentile, II group – 25-75 percentile, and III – 75 percentile. On the basis of these calculations, we proposed the bull-calves with the toxic load coefficient below 10.50 mmol/kg to be assigned to the group of animals with a high potential for weight growth; the bull-calves with a coefficient of 10.51-24.00 mmol/kg to be assigned to the group with average potential; and the bull-calves with a coefficient above 24.01 mmol/kg to be assigned to a group with a low potential for weight growth.

The verification of the reliability of this method was carried out in the conditions of the farm of the Orenburg region, Russia, on physiologically healthy bull-calves (n = 100) of the Kalmyk breed, from which wool samples were taken at 8 months of age when transferred to the feedlot (October), to study the content of Al, Cd, Hg, Pb, Sn, Sr in it. Based on the results, the total toxic load of the body was calculated, and the bull-calves were divided into 3 groups: I (n = 20) \( \sum_{\text{tox}} \geq 24.01 \) units, II (n = 52) \( 10.51-24.00 \) units, and III (n = 28) \( \sum_{\text{tox}} \leq 10.50 \) units. The weighing results showed significant differences in live weight and average daily gain of bull-calves among the compared groups (Table 4).

### Table 4. Performance indicators of the experiment bull-calves from 8 to 18 months of age (M±STD*)

| Age, months | Group I (n=20, \( \sum_{\text{tox}} \geq 24.01 \) mmol/kg) | Group II (n=52, \( 10.51-24.00 \) mmol/kg) | Group III (n=28, \( \sum_{\text{tox}} \leq 10.50 \) mmol/kg) |
|-------------|------------------------------------------------|---------------------------------|---------------------------------|
| Live weight, kg | 211.8±10.88 | 211.1±11.70 | 211.6±11.97 |
| 8 | 211.1±11.70 | 270.2±12.70 | 271.1±13.32 |
| 10 | 317.6±15.18 | 371.8±16.58 | 385.2±19.13 |
| 12 | 434.1±18.86 | 447.2±21.84 | 504.1±23.51 |
| 14 | 488.1±22.89 | 504.1±23.51 | 504.1±23.51 |
| 16 | 488.1±22.89 | 504.1±23.51 | 504.1±23.51 |
| 18 | 488.1±22.89 | 504.1±23.51 | 504.1±23.51 |
| Average daily weight gain, g | 890.0±64.1 | 985.0±67.9** | 991.7±81.4*** |
| 8-10 | 770.5±56.7 | 777.0±64.8 | 918.0±67.7*** |
| 10-12 | 885.0±70.3 | 903.3±69.7 | 968.3±67.1*** |
| 12-14 | 913.3±69.2 | 1038.3±67.1*** | 1033.3±77.9*** |
| 14-16 | 746.7±71.3 | 900.0±76.2*** | 948.3±64.0*** |
| 16-18 | 840.9±55.9 | 920.3±62.2*** | 971.8±69.6*** |

* \( P \leq 0.05; ** P \leq 0.01, *** P \leq 0.001 \) compared to group I

The difference in live weight at 18 months of age was 23.2 kg between groups I-II (5.0%, \( P \leq 0.01 \)), I-III – 39.2 kg (8.4%, \( P \leq 0.001 \)), II-III – 16 kg (3.3%, \( P \leq 0.05 \)); by average daily gains over the period of experiment (8-18 months): I-II groups – 79 g (9.4%, \( P \leq 0.001 \)), I-III – 131 g (15.6%, \( P \leq 0.001 \)), II-III – 52 g (5.6%, \( P \leq 0.001 \)).

### 4. Discussion

Polymorphism of bGH gene (rs135322669) is conditioned by the replacement of 127 amino acid leucine with valine and is associated with different concentrations of growth hormone [13]. Growth hormone (GH) has a very broad biological functionality – it is the peptide hormone of the anterior
pituitary gland, which plays a major role in metabolic processes, helps to increase muscle mass [14] and reduce the amount of fat in the body [15, 16].

Given that the polymorphism of bGH gene changes the production of growth hormone, we studied its effect on the value of the exchange pools of chemical elements in the body. In the course of the study, we did not find significant variations in the elemental status of the estimated micropopulation [17]. It has been established that the exchange pool of macroelements Ca, K, Na and essential trace elements Cu, Fe, J, Se is increased in animals with the CC genotype. It is known that these elements are activators of many tissue metabolic processes, nutrition, regulation of cell growth and differentiation [18, 19] at a reduced concentration of toxic elements Al, Pb, Hg when compared with the genotypes CG and GG.

A decrease in toxic elements with antagonistic bonds may have occurred due to an increase in the concentration of Se [20, 21].

It has been established that exposure to toxic microelements causes a wide range of adverse health effects leading to a decrease in both productive and reproductive qualities of animals [22, 23]. Moreover, against the background of a relatively small consumption of toxic elements [24, 25].

In this regard, it can be assumed that bulls with the CC genotype will not only grow better and be a good product, but also accumulate less toxic elements in the body. In order to confirm this, we will continue with a mandatory study of the animal's productive qualities and safety assessment of the resulting meat products.

5. Conclusion
Studies indicate significant differences in the elemental status of animals depending on the polymorphism of bGH gene. The elemental status of bull-calves with the CC genotype is distinguished by a larger exchange pool of Ca, K, Na, Co, Cr, Cu, J, Se, B, Si, Li, V compared with the genotype CG and of Ca, K, Na, J, Se, B, Li in comparison with GG genotype.

CC genotypes to GG accumulate different toxic elements: Al, Pb, Hg. A technology has been proposed for detecting highly productive animals based on the selection of bull-calves by the level of toxic elements in wool, calculated on the basis of the sum of mmol: Al, Cd, Hg, Pb, Sn, Sr at 8 months of age.

Further research is needed to evaluate the productive qualities of bull-calves of various genotypes and safety characteristics of the products obtained.

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