Influence of growth hormone gene polymorphism on the productive qualities and the level of toxic elements in the hair of Kalmyk breed calves

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Abstract. The studies were performed on a model of calves of the Kalmyk breed (n = 100) from birth to 14 months of age. The effect of growth hormone polymorphism (rs135322669) on productive qualities, body parameters, and toxic load level is estimated. Blood samples were taken to detect gene polymorphism. DNA samples were isolated from whole blood using a DNA-Extran-1 reagent kit. Primers were developed based on published bGH sequences (GenBank Accession NOS. M57764) using Primer3 software. Real-time PCR was performed on an ANK-32 programmable amplifier. The frequency of occurrence of polymorphism in the calves was revealed: 62 % with the CC genotype, 26 % with CG and 12 % with GG. The study of the growth rate of the studied animals from birth to 14 months of age was carried out on the basis of individual monthly weighing. The development was studied on the basis of taking measurements: height at the withers, height at the sacrum, width of the chest, depth of the chest, length of the body, width at the sacrum at 14 months of age. The superiority of calves with CC genotype in live weight starting from 6 months of age over peers with genotypes CG and GG was established. At the age of 6 months it was 5.0–7.0 %, and at 14 months of age 5.0–9.0 %. Calves with the GG genotype were inferior to peers with the CC genotype in height at the withers by 2.3 %, by 2.0 % in the sacrum, by 4.7 % in the chest width, by 5.3 % in the width at the sacrum, by 3.1 % in the chest depth and were inferior to the calves with genotype С by the width of the chest by 2.3 %. The determination of toxic elements: Al, Cd, Pb, Sn, Hg, Sr, was carried out by atomic emission and mass spectrometry (AES-ICP and MS-ICP). Calves with the SS genotype accumulated less toxic substances in the hair from the withers, so their \( \sum_{\text{tox}} \) was 52.4–63.1 % lower as compared to their peers with the CG and GG genotypes. This is also confirmed by the correlation analysis, which revealed a reliable relationship between gene polymorphism and \( \sum_{\text{tox}} \) in wool at the level of \( r = 0.92 \).

1. Introduction
The use of DNA markers in animal breeding is necessary to identify the genes that underlie quantitative traits, while the study of genetic polymorphisms allows for targeted selection with improved indicators of growth intensity, feed use efficiency, carcass quality, etc. [1–4]. The growth process of animals is regulated by many physiological factors, including genes that act in the somatotropic axis, responsible for postnatal growth, the main of which is the growth hormone gene [5, 6].

The bovine growth hormone gene (bGH) is located on the 19th chromosome and includes 5 exons and 4 introns encoding 217 amino acids [7, 8]. Growth hormone, is secreted in the cells of the anterior pituitary of mammals and regulates the expression of genes encoding insulin-like growth factor I
(IGF-I), affects health, milk production, and the growth rate of bones, muscles and adipose tissue [1, 9–11].

Toxic chemical elements in the environment are non-biodegradable pollutants that cause health problems and reduce the productivity of farm animals [12, 13].

It is logical that a high level of toxic chemical elements in the body of farm animals leads to a decrease in metabolic efficiency and a drop in productivity [14–16].

In this case, the assessment of the toxic load should take into account not only individual chemical elements, but also their complexes in view of the close connection between the exchange [17] and the potentiating action of some toxic elements by others [18].

In this regard, studies aimed at studying the effect of growth hormone gene polymorphism on productive qualities, body parameters and the level of general toxic load seem to be promising.

**Purpose of the study.** Identification of the growth hormone gene polymorphism (rs135322669) in the group of calves of the Kalmyk breed and its effect on the productivity and level of toxic elements in the body.

2. **Materials and Methods**

2.1. **Object of study.** Calves of Kalmyk breed

Animal service and experimental studies were carried out in accordance with the instructions and recommendations of the Order of the Ministry of Health of the USSR dated July 27, 1978 No. 701 "On Amendments to the Order of the Ministry of Health of the USSR dated 12.08.77 No. 755" and "The Guide for Care and Use of Laboratory Animals "(National Academy Press, Washington, DC 1996). In carrying out the work, efforts were made to minimize animal suffering and reduce the number of samples used.

2.2. **Experiment design**

The study was conducted in 2020 under the conditions of the SEC of the Krasnogorsk collective farm of the Orenburg region, Russia. To identify one nucleotide polymorphism (SNP) (C / G, rs135322669), blood samples were taken from calves of the Kalmyk breed at the age of 12 months (n = 100). Blood samples were taken in the morning before feeding and drinking. Blood was taken from the caudal vein at the level of the middle third of the body of 2-5 caudal vertebrae into vacuum tubes.

The study of the growth rate of the studied animals from birth to 14 months of age was carried out on the basis of individual monthly weighing. The development was studied on the basis of taking measurements: height at the withers, height at the sacrum, width of the chest, depth of the chest, length of the body, width at the sacrum at 14 months of age. The measurements were taken with a Lidtin measuring stick, a Wilkens compass and a measuring tape.

The wool was sampled from the upper part of the withers of 12 animals goals from each identified group of animals (CC, CG, GG) using a Heiniger Saphir wireless machine (Switzerland) in an amount of not less than 0.4 g according to the procedure [19].

2.3. **Blood sampling and examination**

DNA samples were obtained from whole blood. The DNA isolation protocol was carried out in accordance with the instructions of the commercial kit for the isolation of genomic DNA from whole 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 minus 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 access | G/C          | (F)GGGGGTATGAGAAGCTG     |
|      |          |          | rs135322669   |              | AAGGACCTG                 |
|      |          |          |               |              | (R)CAGGAGCTGGAAAGATGG    |
|      |          |          |               |              | CACGACAC[20]              |

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

Table 2. DNA amplification scheme

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

2.4. Study of wool samples

The elemental composition of the wool was determined by atomic emission and mass spectrometry (AES-ICP and MS-ISP) in the testing laboratory of ANO "Center for Biotic Medicine", Moscow (Registration Certificate of ISO 9001: 2000, Number 4017 – 5.04.06). Biosubstrate ashing 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 total level of toxic elements was calculated by the sum of the amount of the substance of six elements, mmoles: Al, Cd, Pb, Sn, Hg, Sr.

2.5. Statistical processing.

To test the hypothesis of normal distribution of quantitative traits, the Shapiro-Wilk test was used. 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). In all statistical analysis procedures, the achieved significance level (p) was calculated, while the critical significance level in this study was assumed to be less than or equal to 0.05. For data processing, we used the Statistica 10.0 application package (Stat Soft Inc., USA). The tables show the average values of indicators (M) and their standard deviations (± STD).

3. Results

The determination of a single nucleotide polymorphism in the bovine growth hormone gene showed a different frequency of its occurrence in a total sample of 100 calves; were found 62 animals with the CC genotype, 26 animals with CG genotype and 12 animals with GG genotype.

A study of the productive qualities of calves in terms of growth intensity revealed significant differences between the compared genotypes (Table 3).

Calves with the CC genotype starting from 6 months of age were significantly superior to peers with the genotypes CG and GG in live weight. So, at the age of 6 months, their superiority was 7.0 (P≤0.01) and 5.0 % (P≤0.05), 7.4 % (P≤0.01) and 5.7 % (P≤0.05) at 8 months, 8.4 % (P≤0.001) and 4.5 (P≤0.05) % at 12 months and 9.0 (P≤0.001) and 5.0 (P≤0.01) % at 14 months, respectively.

Animals of the same live weight often have different body parameters, the determination of which allows for targeted selective selection. In our study, the features of the exterior formation of calves were studied depending on the polymorphism in the bGH gene (Table 4).
Table 3. Change in live weight of calves of different genotypes for the bGH gene, kg

| Age [months] | Genotype CC (n=62) | Genotype CG (n=26) | Genotype GG (n=12) |
|--------------|------------------|------------------|------------------|
| At birth     | 26.8±2.01        | 26.7±1.63        | 26.3±1.67        |
| 3            | 96.2±7.05        | 93.4±5.65        | 92.1±4.96        |
| 6            | 170.9±9.30       | 162.8±9.06\(^a\) | 159.7±8.21\(^b\) |
| 8            | 225.7±13.17      | 213.6±12.43\(^a\) | 210.2±11.97\(^b\) |
| 10           | 280.4±14.25      | 266.3±13.94\(^a\) | 260.1±13.21\(^b\) |
| 12           | 336.6±16.31      | 332.1±15.96\(^a\) | 310.4±15.72\(^bc\) |
| 14           | 396.8±18.67      | 378.0±17.20\(^a\) | 364.1±16.69\(^bc\) |

\(^a\) – \(P \leq 0.05\) – CG compared with CC;
\(^b\) – \(P \leq 0.05\) – GG compared with CC;
\(^c\) – \(P \leq 0.05\) – GG compared with CG

Table 4. Connection of bGH gene polymorphism with body measurements of calves at the age of 14 months, cm

| Measurement                  | Genotype CC (n=62) | Genotype CG (n=26) | Genotype GG (n=12) |
|------------------------------|------------------|------------------|------------------|
| Height at the withers        | 120.2±2.75       | 118.9±3.18       | 117.4±2.48\(^a\) |
| Sacral height                | 123.3±2.59       | 121.7±2.83       | 120.8±2.05\(^a\) |
| Chest width                  | 40.1±1.43        | 39.1±1.24        | 38.2±1.32\(^ab\) |
| Chest depth                  | 61.1±1.32        | 60.3±1.47        | 59.2±1.20\(^a\) |
| Torso length                 | 141.4±3.72       | 140.4±3.80       | 139.3±3.14       |
| Sacrum Width                 | 43.4±1.70        | 42.2±1.82        | 41.1±1.20\(^a\) |

\(^a\) – \(P \leq 0.05\) – GG compared with CC;
\(^b\) – \(P \leq 0.05\) – GG compared with CG

Calves with the GG genotype were inferior to peers with the CC genotype both in height measurements (by 2.3 % \((P \leq 0.01)\) at the withers, by 2.0 % \((P \leq 0.01)\) at the sacrum), and width measurements (by 4.7 % \((P \leq 0.001)\) in chest width, by 5.3 % \((P \leq 0.001)\) in the width at the sacrum, by 3.1 % \((P \leq 0.001)\) in the chest depth. To genotype CG, they were inferior only in width breast by 2.3 % \((P \leq 0.05)\).

To assess the total toxic load of the body of calves depending on the polymorphism of the growth hormone gene for each group, the \(\Sigma_{\text{tox}}\) concentrations were calculated as the sum of the mmoles of elements: Al, Cd, Pb, Sn, Hg, Sr in the hair from the withers (Fig. 1).

Calves with the CC genotype, having a high growth rate, accumulated less toxic substances in the hair from the withers, so their \(\Sigma_{\text{tox}}\) was lower by 52.4 \((P \leq 0.001)\) and 63.1 % \((P \leq 0.001)\) in comparison with peers with genotypes CG and GG, respectively. This is also confirmed by the correlation analysis which revealed a reliable relationship between the polymorphism of the gene and \(\Sigma_{\text{tox}}\) in the coat at the level of \(r = 0.92\).

4. Discussion

More than 35 years ago, a library of cloned cattle DNA fragments was created. The growth hormone gene was isolated from this library with the determination of its nucleotide sequence [21]. From this moment begins an intensive study of its effect on the body. As is known today, the gene for growth hormone (GH) is considered as an important element in the control of human growth and development [22], cattle [23, 24], pigs [25], fish [26], etc.

This study shows that SNP (rs135322669) in the bGH gene is associated with changes in body weight and body proportions in calves of the Kalmyk breed. Mutations of a gene with CC (leucine /
(leucine) to GG (valine / valine) at a low frequency of occurrence (12.0 %) were accompanied by a decrease in both height measurements: height at the withers, in the sacrum, and latitudinal ones: width and depth of the chest, the width at the sacrum, which led to a difference of 14 months in live weight between the compared genotypes in 9.0 % (P (0.001). This does not contradict previous studies [27, 28].

\[ \text{Figure 1. Concentration of the sum of toxic elements (} \Sigma_{\text{tox}} \text{) in calves of different genotypes, mmol/kg} \]

The revealed dependence of the bGH gene polymorphism and the sum of toxic microelements is difficult to analyze and requires additional studies.

5. Conclusion
The studies indicate the influence of polymorphism in the bGH gene of calves of Kalmyk breed on the parameters of their body: height at the withers and sacrum, width and depth of the chest, width in the sacrum.

Calves with the CC genotype starting from 6 months of age are significantly superior to peers with the CC and CG genotypes in live weight.

The data on the total toxic load of the body of calves, depending on the polymorphism of the growth hormone gene, indicate its significant difference with reliable correlation (r = 0.92).

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