Reducing the modifying impact of the ecological factor at breeding value assessment of Hereford bull calves taking into account phenotype and complex of genes GH and GDF5

K M Dzhulamanov, S V Lebedev, N P Gerasimov and E B Dzhulamanov
Federal Scientific Center of Biological Systems and Agricultural Technologies of the Russian Academy of Sciences, Orenburg

E-mail: kinispai.d@yandex.ru

Abstract. The objective assessment of beef cattle genetic potential is a priority task of beef cattle breeding intensification. The purpose of the study was to test a method for reducing the modifying effect of ecological factor in assessing the breeding value of Hereford bulls based on combined selection, taking into account the phenotype assessment and genotyping by GH and GDF5 genes. For this purpose, the bull calves were tested according to productivity in two variants: 1) setting and selection of young cattle for control cultivation on the basis of phenotype (traditional assessment); 2) traditional selection was supplemented by genotyping assay for the complex of GH and GDF5 genes. The individuals with desired allelic profile (genotypes VV and VL of GH gene and genotypes TT and TC of GDF5 gene) showed increased weight gain intensity relative to their herd mates. The analysis of body weight repeatability data at different stages of ontogenesis shows that the selection of carriers of desirable alleles for the gene complex increases the accuracy of high-priced animal selection. Thus, in order to determine the genetic potential of Hereford bull calves more objectively and minimizing the ecological impact on breeding value, it is advisable to use a combined approach to selection that unites the assessment of the phenotype of young cattle and the genotyping of animals taking into account the complex of genes GH and GDF5.

1. Introduction
The strong dependence of quantitative traits development on limiting environmental factors in beef cattle, as well as frequently changing of distribution ranks of genotypes by productive qualities in different ecological conditions, bring to the fore the problem of genotype-environment interaction [1]. Any change in environmental conditions contributes to ecological variability in the activity of quantitative traits loci. In this regard, the strong mobility of the breeding value limits the possibilities of developing a stable selection and breeding program [2].

The breeding value realization of young beef cattle breeds occurs on the existing factors background of feeding, housing and the environment. At the same time, the animal’s productivity does not always reflect its genetic potential, but is the expression of the genotype under specific rearing conditions. A change in the system of paratypical factors will contribute to the manifestation of the modifying variability in selected traits. The range of variability depends on stability and plasticity of the animal organism. High breeding value of beef cattle is largely determined by the level of animal adaptation to the rearing conditions [3].
Different versions of alleles of the growth differentiation factor-5 gene (GDF5) determine
morphogenesis of bones, ligaments and tendons in humans and animals [4]. The regulation of tissue
growth and differentiation in the body is ensured by the growth hormone [5].

In view of the intensive use of genomic assessment in breeding, which today is one of the most
accurate, it is necessary to focus on a more detailed attitude towards the assessment of breeding bull
calves for the reproduction of high-priced herds [6–7].

The purpose of the study was to test a method for reducing the modifying effect of ecological
factor in assessing the breeding value of Hereford bulls based on combined selection, taking into
account the phenotype assessment and genotyping by GH and GDF5 genes.

2. Materials and methods
The selection of replacement Hereford bull calves was improved in the tribal plant LLC Agrofirma
Kalininskaya of Chelyabinsk region. This was accompanied by stage-by-stage identification of
perspective animals at 8-month age: genealogical → phenotypical → genetic assessment. At the first
stage, the task was to determine the validity of origin of Hereford bull calves. For breeding
assessment, the bull calves were selected from distinguished fathers whose origins were confirmed by
immunogenetic tests, as well as documents of livestock and breeding records. At the next stage, such
phenotypic signs as the body weight and height at hips were studied. The groups of replacement bull
calves were composed of the most massive, high-growth and harmoniously built animals. The final
stage was based on the genotyping of bull calves by GH and GDF5 genes. From the genotyped
population, the total sample (group I) of 40 Hereford bull calves with high phenotypic indicators of
productivity was formed and herdmates (n=12 heads) with the desired allelic profile (group II):
genotypes VV and VL by GH gene and genotype TT and TC by GDF5 gene. Thus, according to DNA
testing, the desired genotypes with higher phenotypic qualities at the age of 8 months (body weight,
the type of constitution by height at hips) were selected and put to the experiment according to their
productivity up to 18 months of age.

For genotyping by growth hormone (GH) and growth differentiation factor (GDF5), the blood
samples from the jugular vein were taken from the bull calves. The whole blood was placed into tubes
with 600 μl of ethylenediaminetetraacetic acid (EDTA) to give a volume of 10 ml.

Genotyping was carried out on the basis of DNA isolated from blood using DIAtom™ DNA Prep
reagents (IsoGeneLab, Moscow) at the laboratory of the Common Use Center of Biological Systems
and Agritechnology RAS (accreditation certificate No. RA.RU.21PF59 dated 29.12.2015). The
GenePakPCRCore sets (IsoGeneLab, Moscow) were used for PCR tests.

In order to evaluate the polymorphism of the GH gene, genotyping was performed by PCR-RFLP
on a programmable Tercik thermocycler (DNA technology, Russia). The primers were used to amplify the sites (Table 1):

| Genetic marker | Primer sequence | Size of amplified fragment, bps |
|---------------|-----------------|------------------------------|
| GH            | F: 5’-gct-gct-cct-gag-ggc-cct-tcg-3’ | 223                          |
|               | R: 5’-gcg-gcg-gca-ctt-cat-gac-ctg-3’ |                              |

PCR program for GH gene: hot start – 5 min. At 95 ºC; 35 cycles: denaturation – 45 sec. at 94 ºC,
annealing – 45 sec. at 65 ºC, synthesis – 45 sec. at 72 ºC; extension – 7 min. at 72 ºC. AluI
endonuclease was used to restrict the amplified regions of the gene. The cleavage of products was
 carried out at 37 ºC, genotypes were identified by gel electrophoresis with imaging under UV light.
Identification of products for the growth hormone gene: GH<sup>VV</sup> – 223 bps; GH<sup>VL</sup> – 223, 171, 52 bps;
GH<sup>LL</sup> – 171, 52 bps.

The nucleotide sequence of the primer for the GDF 5 genetic marker is shown in Table 2.

PCR-RFLP of the GDF5 gene was carried out in a MyCycler thermocycle (BioRad, USA). PCR
protocol: initiating DNA denaturation within 5 min. at 95 ºC, then 32 cycles of amplification,
denaturation at 94 °C (30 s), annealing at 60 °C (30 s) and extension at 72 °C (30 s), final synthesis at 72 °C within 10 min. The restriction reaction of obtained amplification products of GDF5 was carried out with use of MvaI restriction endonuclease (Table 3).

### Table 2. Characteristics of the primer used in the study

| Genetic marker | Primer sequence | Size of amplified fragment, bps | Data source |
|----------------|-----------------|--------------------------------|-------------|
| GDF5           | F: 5’-TGTCGATGCTGACAGAAAGG-3’<br>R: 5’-GAGTGAGGTTAATCCCAGATACCA-3’ | 235 | Liu Y.F. et al. (2010) |

In total, 20 μl of PCR product and 10 MvaI units were mixed in a tube for the reaction followed by incubation at t=37 °C within 5 hours. The resulting product was separated by horizontal electrophoresis (in 1x tris-borate buffer at 80 V) in a 2.5 % agarose gel with ethidium bromide staining. The gel was then analyzed in ultraviolet light on a UVT-1 transilluminator, photographed using VITran v.1.0 system. The length of fragments was determined using the molecular weight marker GenePakR DNA Ladder M 50 (IsoGene Lab, Moscow).

### Statistical processing

Microsoft Office software with Excel (Microsoft, USA) and Statistica 10.0 (Stat Soft Inc., USA) was used to process the experimental data with variation and correlation analysis. The statistical significance of differences between the groups was assessed by Post-hoc Tukey’s HSD test for unequal N.

### Results and discussion

The study based on polymerase chain reaction – restriction fragment length polymorphisms (PCR-RFLP) revealed single nucleotide polymorphism (SNP) in the position of T586C exon 1 in GDF5 and in the position of C2141G of exon 5 of GH gene when genotyping 149 heads of Hereford bull calves (Table 4).

### Table 4. Distribution of Hereford bull calves (n=149 heads) by GDF5 and GH genotypes, %

| GDF5 genotype | VV | LV | LL | Total |
|---------------|----|----|----|-------|
| TT            | 0.67 | 1.34 | 5.37 | 7.38 |
| CT            | 2.01 | 4.03 | 6.04 | 12.08 |
| CC            | 17.45 | 24.83 | 38.26 | 80.54 |
| Total         | 20.13 | 30.20 | 49.66 | 100.0 |

Note: carriers of desirable alleles are marked grey

The analysis of obtained data shows a significant increase in the selection intensity of replacement bull calves. Thus, of the 149 heads with confirmed breeding record, 40 animals (26.8%) were assessed phenotypically. While the combined approach with the identification of desirable allelic variants of GDF5 and GH genes increased the selection pressure resulting in only 12 heads (8.05%) satisfying the dependent selection marker.

Being under identical ecological conditions (the system of rearing and feeding) the bull calves with different allelic profile had some differences in the body weight (Table 5).

At 8 months of age, the bull calves marked with desirable alleles of GDF5 and GH genes outperformed their herdmates in the general group with alternative genotypes (group I) by 11.2 kg (4.8 %; P>0.05). At the age of 15 months, the intergenic rank of animals in terms of the body weight
remained the same as in the previous age period. Thus, the advantage of group II bull calves over their intrabreed herdmates from group I was 22.5 kg (5.0 %; P<0.05).

Table 5. Change of the weight growth of Hereford bull calves depending on complex genotype GH and GDF5 (X±Sx)

| Age period, months | Group | I | II       |
|--------------------|-------|---|----------|
|                    | Body weight, kg | 234.0±2.62 | 245.2±4.92 |
| 8                  | 15                | 451.4±3.99 | 473.9±5.77* |
| 18                 |                   | 534.7±4.90 | 573.9±9.72** |
|                    | Average daily gain, g | 1021.0±11.38 | 1073.9±11.69 |
| 8-15               |                   | 914.6±24.61 | 1098.9±48.36** |
| 15-18              |                   | 989.1±11.98 | 1081.4±19.39** |

Note: * – validity of difference P<0.05; ** – P<0.01.

The study of meat livestock primarily dealt with the increase of the body weight. It is common knowledge that even at any grab stage of growth, the final meat product is influenced by breed, sex, physiological condition, ecological factor. On the basis of the above, we believe that the identification of the nature and sustainability of inheritance of selectable features should be a mandatory element in assessing the breeding qualities of bull calves and stud bulls. Besides, one of the main properties in the selection of stirps and continuer of genealogical lines is the guaranteed increase in long-life and body weight of animals of most meat breeds and adaptability to existing ecological conditions. Therefore, in the same 15-month-old Hereford bull calves, the duration of the test period was extended by 3 months. Obviously, in order to justify the selection of replacement animals for further use, it was necessary to clarify (increase the reliability) the results of the previous assessment.

With age the intergroup differences in the body weight acquired even more contrasting forms. The superiority of the 18-month-old bull calves marked with the desirable alleles of GDF5 and GH genes compared to their herdmates with alternative genotypes was 39.2 kg (7.3 %; P<0.01).

The differences in the body weight are due to different growth intensity of bull calves, which is determined by the genetic potential and the organism reaction to the ecological conditions of rearing. At the same time the rank of distribution of animals by the value of the studied indicator was similar to that by weight. In a more detailed examination at certain technological stages of cultivation and by age period of the average daily gain of the body weight, it was found that the bull calves marked with desirable alleles of GDF5 and GH genes did not decrease the superiority on this selection feature over the herdmates with alternative genotypes.

Between 8 and 15 months they had the superiority over the bull calves of group I by 52.9 g (5.2 %; P<0.05). In the following 15–18 months this superiority increased to 184 g (20.1 %; P<0.01). The largest absolute gain in the body weight was recorded in the carriers of desirable V and T alleles by genotypes VV and LV of GH gene, and TT and CT of GDF5 gene. This confirms the use of DNA markers to objectively evaluate the breeding bull calves by long-growing ability and considerable weight as well as reducing the uncontrolled impact of ecological factor on the implementation of the genetic potential.

The high selection significance of the DNA marker method for determining the breeding value of the animals by their own productivity is confirmed by continued performance of the previous monitoring after the extension of the test period.

In studying the repeatability of selectable features (Table 6) at different technological stages, it was found that genotyping of replacement bull calves by GH and GDF5 genes and selection of carriers of desirable alleles by the gene complex increases the accuracy of selection of high-priced animals. At the same time, there is a possibility of assessing the selection of Hereford bull calves at early stages of their development.
Table 6. Repeatability of weight growth indicators

| Age period, months | Group I | Group II |
|--------------------|---------|----------|
| 8-12               | 0.20±0.159 | 0.97±0.077*** |
| 8-15               | 0.81±0.095*** | 0.90±0.138*** |
| 8-18               | 0.69±0.117*** | 0.88±0.150*** |
| 12-15              | 0.03±0.162 | 0.96±0.089*** |
| 12-18              | 0.04±0.162 | 0.93±0.116*** |
| 15-18              | 0.89±0.074*** | 0.97±0.077*** |

Thus, the analysis of the obtained data shows that the ranking of the population taking into account the allelic profile increases the degree of correspondence between productivity indicators of bull calves at different ages. The animals’ selection only by phenotype does not guarantee the achieved level of trait development when re-evaluating at the next technological stage due to changing ecological conditions of rearing.

4. Conclusion

The genetic structure of the Hereford herd in Chelyabinsk region is represented by nine genotype variants of GH/GDF5 complex associated with weight and linear growth of animals. Four genetic constructs include desirable alleles in both genes, the share of carriers of which is 8% in the population. Individuals with the desired allelic profile exhibited increased weight growth intensity relative to herdmates selected only by phenotype (body weight + expression of constitutional type). Thus, in order to determine the genetic potential of Hereford bull calves more objectively and reducing the modifying effect of the ecological factor, it is advisable to use a combined approach to selection thus combining the assessment of the phenotype of bull calves and the genotyping of animals taking into account the GH and GDF5 complex.

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