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Productivity and immune reactivity of small cattle with different genotypes

ABSTRACT

Modern methods of breed formation in animal husbandry underscore the importance of studying the influence of genetic factors on the productive qualities of farm animals. This research analyzes the association between candidate gene polymorphisms and meat productivity indicators and immune status in sheep. Genotyping of the GH, GDF9, and CAST loci was performed using the PCR-RFLP method in a sample of Dagestan Mountain sheep. Statistically significant associations were established between the genetic variants and the traits under study. Animals with the GH/HaeIII AB and GDF9/AspIEI AA genotypes were characterized by an increased level of CD4+ lymphocytes and a reduced content of CD8+ cells, resulting in higher values of the immunoregulatory index compared to carriers of the GH/HaeIII AA and GDF9/AspIEI GG genotypes. Analysis of productive qualities revealed the advantage of the CAST MM genotype over CAST MN in terms of live weight. A similar pattern was established for the GH AA genotype compared to GH AB. The obtained data demonstrate the promise of using molecular genetic markers in breeding programs for the simultaneous improvement of both productive characteristics and the immune status of sheep.

Key words: marker selection, genotyping, small ruminants, immune reactivity, productivity

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Научная статья

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Продуктивность и иммунная реактивность мелкого рогатого скота разных генотипов

РЕЗЮМЕ

Современные методы породообразования в животноводстве актуализируют задачу изучения влияния генетических факторов на продуктивные качества сельскохозяйственных животных. В рамках настоящего исследования проведен анализ взаимосвязи полиморфизма генов-кандидатов с показателями мясной продуктивности и иммунного статуса у овец. Методом ПЦР-ПДРФ осуществлено генотипирование по локусам GH, GDF9 и CAST в выборке горных дагестанских овец. Установлены статистически значимые ассоциации между генетическими вариантами и изучаемыми признаками. Животные с генотипами GH/HaeIII AB и GDF9/AspIEIAA характеризовались повышенным уровнем CD4+-лимфоцитов и сниженным содержанием CD8+-клеток, что обусловило более высокие значения иммунорегуляторного индекса по сравнению с носителями генотипов GH/HaeIII AA и GDF9/AspIEIGG. В ходе анализа продуктивных качеств выявлено преимущество генотипа CASTMM над CASTMN по показателям живой массы. Аналогичная закономерность установлена для генотипа GHAA в сравнении с GHAB. Полученные данные демонстрируют перспективность использования молекулярно-генетических маркеров в селекционных программах для одновременного улучшения продуктивных характеристик и укрепления иммунного статуса овец.

Ключевые слова: селекция маркеров, генотипирование, мелкий рогатый скот, иммунная реактивность, продуктивность

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Introduction/Введение

In sheep breeding, a key priority is the assessment and improvement of productivity, which depends on both genetic and environmental factors [1–5]. Breeding approaches that account for these factors are widely used in agricultural production and have contributed to better meat quality [6–9]. In practice, slaughter evaluation provides core indicators such as dressing percentage and carcass weight, which can correlate with specific genotypes [10–14]. Combining traditional methods with molecular tools, including genetic markers, enables systematic analysis of genetic resources and accelerates breeding programs[1] [15, 16]. Because many economically important traits are complex, results across breeds can be heterogeneous; continued study is needed to refine gene–trait links, manage genetic diversity, and improve prediction of productivity [17–21].

Maintaining diversity is also essential to avoid inbreeding depression under intensive selection schemes [22, 23]. For effective use of selection tools, the genetic structure of local populations must be described [24]. Identifying associations between gene variants and meat-production traits enables development of assays and, ultimately, genomic selection pipelines [25–28].

The Dagestan Mountain (DM) sheep and several other regionalized breeds remain understudied in this respect [29–31]. Marker-assisted selection (MAS) in sheep increasingly relies on well-characterized candidate genes. Polymorphisms in GH, CAST (calpastatin), and GDF9 (growth differentiation factor 9) have been extensively studied across breeds, with reported associations to growth, carcass/tenderness, and reproductive traits (GH [31–34], CAST [35–37], GDF9 [38–40]).

Building on this evidence, our study quantifies allele/genotype frequencies in [population] and tests genotype–phenotype associations relevant for selection. Improving economically important traits in DM sheep while conserving breed specificity is expected to raise meat quality and sustain adaptability to harsh local environments. Introducing validated DNA markers into selection schemes for DM sheep and relevant crossbreds can increase selection efficiency and positively impact production metrics [41, 42].

Despite extensive evidence for GH, CAST and GDF9, data for Dagestan Mountain sheep and regional crosses are lacking. Given the breed's economic role and the need for rapid on-farm decisions, locally validated marker-trait associations are required to de-risk selection and improve prediction of growth and carcass outcomes. Therefore, we aimed to validate polymorphisms in GH, CAST, and GDF9 in DM sheep and crosses, quantify their associations with growth, carcass, and immune traits, and derive selection-ready recommendations for marker-assisted decisions under farm conditions.

Materials and methods / Материалы и методы исследования

The study was conducted at Agrofirma "Sogratl", Gunibsky District, Republic of Dagestan, Russia, during 2022–2023 under lowland and mountainous grazing. The cohort comprised 114 crossbred sheep (Dagestan Mountain × Russian Meat Merino; 85 ewes, 29 rams) maintained under comparable feeding and housing.

Observational design to evaluate genotype-phenotype associations relevant for selection. Primary factor (fixed effect): genotype at candidate loci (GH, CAST, GDF9); levels used in analyses: GH (AA, AB), CAST (MM, MN), GDF9 (AA, AG, GG). Covariates: sex; age or measurement stage (where applicable).

Primary outcomes: longitudinal live weight; carcass traits (slaughter subset); immune cell subsets with immune-regulatory index (IRI = $CD4^{+}/CD8^{+}$). Reporting of genetic associations aligns with STREGA. Jugular venous blood (5–10 ml) was collected aseptically into heparinized vacutainers (25 U/ml). Samples were stored at 4 °C for up to 24 h or frozen at — 20 °C until DNA extraction. Genomic DNA was isolated with DIAtom™ DNAPrep (IsoGeneLab, Moscow); typical yield 3–5 μg/100 μl, A260/280 = 1.6–2.0. Three loci were genotyped by PCR-RFLP: GH (HaeIII), CAST (MspI), GDF9 (BstHHI).

Primer pairs/annealing temperatures and expected amplicons are summarized in Table 1. PCRs were performed in 20–25 μl reactions (1 × buffer, 1.5–2.0 mM $MgCl_2$, 0.2 mM each dNTP, 0.4 μM each primer, 1 U Taq polymerase) with cycling 95 °C 3 min; 35 cycles of 95 °C 30 s, annealing 60–63 °C 30 s, 72 °C 30 s; final 72 °C 5 min. Amplicons were digested with the specified endonucleases and resolved on 2–3% agarose; two independent readers called genotypes from fragment patterns [34].

The samples were stored at 4 °C for up to 24 hours or frozen at — 20 °C until DNA extraction for PCR-RFLP genotyping of GH, GDF9, and CAST genes Table 1.

The experiment was conducted in compliance with the requirements set out in Directive of the European Parliament and of the Council of the European Union of 22 September 2010 No.. 2010/63/EU on the protection of animals used for scientific purposes and the principles[2] of treatment of animals in accordance with Article 4 of Federal Law No. 498-FZ4[3].

[1] Оценка изменчивости генов, ассоциированных с живой массой и шерстной продуктивностью овец: научно-методические рекомендации / Г.М. Гончаренко, Т.Н. Хамируев, С.М. Дашинимаев *и др.* Чита: Институт развития образования Забайкальского края. 2024; 35. ISBN 978-5-6045472-6-7, EDN WJGWSL

[2] Directive of the European Parliament and of the Council the European Union On the protection of animals used for scientific purposes. https://ruslasa.ru/wp-content/uploads/2017/06/Directive_201063_rus.pdf

[3] Федеральный закон от 20.12.2018 № 498-ФЗ (ред. от 24.07.2023) «Об ответственном обращении с животными и о внесении изменений в отдельные законодательные акты Российской Федерации».

Table 1. **Primers and PCR-RFLP conditions for candidate loci**

Gene	Nucleotide sequences	T (°C), Annealing	Amplicon, bp	Endonuclease	Genotypes
CAST	F:5'-tggggcccaatgacgccatcgatg-3' R:5'-ggtggagcacttctgatcacc-3'	62	422	MspI	MM/MN
GH	F:5'-ggaggcaggaagggatgaa-3' R:5'-ccaagggagggagagacaga-3'	60	277	HaeIII	AA/AB/BB
GDF9	F:5'-gaagactggtatggggaaatg-3' R:5'-ccaatctgctcctacacacct-3'	63	462	BstHHI	AA/AG/GG

Cellular immunity was assessed in carriers of selected GH and GDF9 genotypes by flow cytometry. Whole blood (EDTA) was stained with fluorochrome-conjugated monoclonal antibodies to CD3, CD4, CD8, and CD21 with appropriate isotype controls; erythrocytes were lysed with a commercial kit. Data were acquired on an Attune™ NxT Acoustic Focusing Flow Cytometer (Thermo Fisher Scientific, USA), a benchtop analyzer that uses acoustic-focusing fluidics; the system supports configurations with four lasers (405/488/561/637 nm) and up to 14 colors, and runs Attune Cytometric Software for acquisition and analysis.

Individual live weight was recorded at predefined stages birth, pre-transhumance, arrival on mountain pastures, weaning, pre-winter, arrival on winter pastures) using low-profile platform livestock scales Promves-PL-VZh (Promvesoborudovanie LLC, Yekaterinburg, Russia) — a non-automatic weighing instrument, OIML R 76 Class III, configured for static weighing with strain-gauge load cells. The model series is listed in the Russian State Register of Measuring Instruments (entry No. 87205-22; valid until 24 Oct 2027; inter-verification interval 1 year).

The farm configuration provided capacity 600–2000 kg (series options up to 40 000 kg), graduation 0.1–0.2 kg, and a 1000 × 2000 mm platform. Before each weighing session, the scale was checked with certified standard weights; two consecutive readings were averaged (difference ≤ 0.2 kg). Measurements adhered to the principles for non-automatic weighing instruments in OIML R 76; for national practice we also reference GOST 25955-83[4] (methods for determining sheep productivity parameters). All animals were clinically healthy, and the conditions of their maintenance complied with zootechnical standards and zoohygienic requirements[5].

Analyses were performed in R (v4.x) using lme4 for mixed models and emmeans for estimated marginal means; HardyWeinberg was used for Hardy — Weinberg tests. Live weight (repeated measures) was analysed with a linear mixed model weight — genotype + stage + + genotype: stage + sex + (1|animal); we report estimated marginal means (EMMs) with Tukey multiplicity adjustment for within-factor contrasts, effects (β), 95% CIs and two-sided P. Immunology traits were modelled as trait — genotype + sex + age + + (1|batch); normality and homoscedasticity were checked on residuals, with log-transform or a GLMM alternative if assumptions were violated; EMMs are reported with multiplicity control.

Carcass traits were analysed with linear (or mixed) models trait — genotype + sex; given the small slaughter subset, we emphasised effect sizes with 95% CIs and used robust / permution-based checks if normality was untenable. For genotypes, allele/genotype frequencies were computed and HWE was assessed per locus using the exact test (Hardy — Weinberg — HWExact) with a χ^2 sensitivity check; rare, unobserved levels (e. g., CAST NN) are explicitly documented. Across families of outcomes we additionally controlled the false-discovery rate (BH-FDR, $q < 0.05$). Factor coding and reference levels are specified in the Results/Supplement. Reporting of design, sample size, and statistics follows ARRIVE 2.0 and STREGA recommendations.

Results and discussion / Результаты и обсуждение

The importance of the immune system for growth and development is especially relevant during the growth period of lambs, since it is during this period that the immune status is established.

The study of the levels of T — and B-lymphocytes in the blood of lambs of different genotypes made it possible to assess their immune potential Table 2.

It was found, that the Dagestan Mountain lambs with the genotypes GH/HaeIII AB and GDF9/AspIEIAA had a higher level of T-helpers and lower T-suppressors,

Table 2. **Indicators of immune reactivity of lambs of different genotypes**

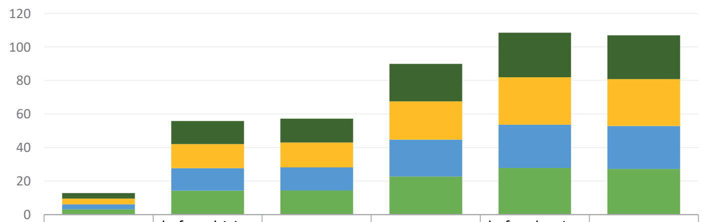
Genotypes by genes	Indicator				
	Immune reactivity, 10^9/l				*IRI*
	T-cells	*B-cells*	*T-suppressors*	T-helpers	
Dagestan mountain breed of sheep (n = 30)					
GH^{AA}	0.60 ± 0.09	0.52 ± 0.06	0.34 ± 0.04	0.23 ± 0.16	0.68
GH^{AB}	0.69 ± 0.12	0.53 ± 0.08	0.26 ± 0.15	0.29 ± 0.06	1.1
GDF9^{GG}	0.59 ± 0.08	0.35 ± 0.1	0.40 ± 0.14	0.28 ± 0.14	0.7
GDF9^{AA}	0.66 ± 0.15	0.40 ± 0.14	0.3 ± 0.1	0.35 ± 0.16	1.2
GDF9^{AG}	0.61 ± 0.08	0.35 ± 0.12	0.38 ± 0.12	0.26 ± 0.14	0.68
Crossbred lambs (n = 20)					
GH/HaeIII^{AA}	0.57 ± 0.08	0.42 ± 0.06	0.36 ± 0.14	0.37 ± 0.05	1.03
GH/HaeIII^{AB}	0.62 ± 0.14	0.50 ± 0.12	0.42 ± 0.08	0.38 ± 0.14	0.9
GDF9/AspIEI^{GG}	0.5 ± 0.06	0.42 ± 0.16	0.3 ± 0.04	0.37 ± 0.04	1.23
GDF9/AspIEI^{AA}	0.65 ± 0.12	0.47 ± 0.16	0.32 ± 0.06	0.3 ± 0.02	0.94
GDF9/AspIEI^{AG}	0.54 ± 0.14	0.46 ± 0.06	0.34 ± 0.06	0.35 ± 0.02	1.03

[4] ГОСТ 25955-83 Сельскохозяйственная племенная продуктивность. Методы определения показателей продуктивности овец. М.: Издательство стандартов. 1983; 13.
[5] ГОСТ 34088-2017 Руководство по размещению и уходу за лабораторными животными. Правила содержания и ухода за сельскохозяйственными животными. М.: Межгосударственный совет по стандартизации, метрологии и сертификации. 2018; 36.

which affected on the immunoregulatory index (IRI), making it higher, compared to the lambs of the genotypes GH/HaeIII AA and GDF9/AspIEIGG.

Crossbred lambs also had a higher number of B — and T-lymphocytes with genotypes GH/HaeIII AB and GDF9/AspIEIAA. The studies show, that the body's reactivity on negative environmental influences is specific for each genotype and is apparently determined by an innate genetic program. This program allows to increase production of T — and B-lymphocytes, as well as T-helpers, but decrease the number of T-suppressors. Such regulation helps to correct and strengthen of the adaptation and compensation systems in the body on various stages of its development.

Fig. 1. Live weight indicators of Dagestan Mountain ewe lambs of different genotypes



	At birth	before driving to the mountains (2.5 months)	upon arrival in the mountains (3.0 months)	during beating (4.3 months)	before leaving for winter pastures (5.3 months)	upon arrival at winter pastures (6.0 months)
GHAB	3.2	13.8	14.2	22.4	26.6	26.1
GHAA	3.4	14.4	14.8	22.8	28.2	28
CASTMN	3	13.5	13.7	22	25.9	25.5
CASTMM	3.2	14.2	14.5	22.7	27.8	27.3

With the development of molecular genetics, new opportunities are opening up for optimizing of breeding selection and increasing of the efficiency of livestock farms.

A key aspect is the identification of genotypes, that can improve the productive characteristics of animals. Particular attention is paid to genes, that affect on productive qualities, including the growth hormone gene (GH) and calipain (CAST).

In the context of studying of the effect of genetic markers on the weight of Dagestan Mountain lambs, their weight was measured on various stages of life.

This made it possible to evaluate the relationship between various forms of the CAST and GH genes and changes of live weight (Fig. 1).

Analysis of the sheep weight showed, that the greatest increase of weight was observed before the period of grazing on summer pastures, when the average gain was 14.6 kg. Also, a significant gain 23.0 kg was recorded in the time of separation of mothers from lambs. At the same time, the lowest weight indicators were noted during the period of movement between winter and summer pastures, while their average weight was 27.1 kg.

During the study of various stages of development of experimental individuals, it was revealed, that the highest rates of weight gain were observed in individuals with certain genetic markers. Thus, individuals, carrying a combination of MM on the CAST gene and AA on the GH gene, demonstrated the best results in body weight gain and daily weight gain. It is noted, that the sample of analysis did not include individuals with the CASTNN and GHBB genotypes, which excludes the possibility of their assessment within the framework of the study. The study showed, that animals with the CASTMM genotype have a larger mass on average, compared to their relatives with the CASTMN genotype.

Observations in different periods revealed the following differences in mass: before being taken to pasture, the difference was 4.9%, and when the cattle reached the mountain pastures — 5.5%. After the summer period, during weaning, the gap decreased to 3.1%. However, before being sent to wintering and upon arrival there, the difference increased to 6.9 and 6.6% respectively.

In relation to the GH gene, differences in weight gain were also observed between carriers of the GHAA and GHAB genotypes.

Thus, when grazing on summer pastures, animals with the GHAA genotype had a 4.2% weight advantage. When the cattle reached summer pastures, the weight difference was — 4.8%. The difference between carriers of the AA and AB alleles increased before being sent to wintering and upon arrival there, and amounted to 5.7 and 6.8%, respectively.

The study showed, that certain genotypes, namely GHAA and CASTMM, demonstrate better results in live weight and weight gain, compared to GHAB and CASTMN. The observed differences in productivity become especially noticeable during key periods of cattle breeding, such as the transfer of animals to summer and winter pastures. At critical moments, such as before the cattle are driven to the mountains and back, as well as during of the change of seasonal pastures, the advantage of the GHAA and CASTMM genotypes becomes obvious.

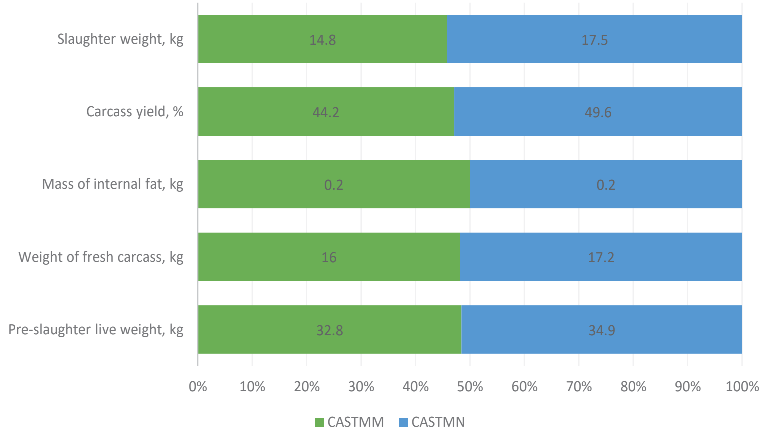
To analyze the quality characteristics of meat of different sheep genotypes, including its composition and nutritional value, control slaughters are carried out. During the study, crossbred rams with the CASTMM and CASTMN genotypes were weighed individually. This allowed us to select animals with similar weights for slaughter. Before the slaughter procedure, specialists examined the sheep to ensure their health and readiness for slaughter.

The study of different genotypes of CASTMM and CASTMN sheep showed interesting differences in their parameters before and after slaughter.

Animals with the CASTMM genotype (Fig. 2) demonstrated better results on number of indicators.

When evaluating of the carcass after slaughter, the difference was also noticeable: CASTMM ewes surpassed CASTMN on 7.0% in terms of fresh carcass weight. While in terms of internal fat content, ewes with the CASTMN and CASTMN genotypes showed equal values. The percentage of carcass weight to live weight was on 10.9% higher in CASTMM, indicating

Fig. 2. Results of control slaughter of crossbred rams with genotypes CASTMM and CASTMN



on their more favorable slaughter qualities. In general, compared to CASTMN, the slaughter weight in ewes with the CASTMM genotype was almost on 15.4% higher, which can play a significant role in commercial breeding.

Conclusions/Выводы

Based on the study results, the CASTMM genotype demonstrates statistically significant advantages for carcass-related traits under the evaluated farm conditions. The GHAA genotype shows a consistent association with pre-weaning growth performance in lambs.

The confirmed marker-trait relationships support the practical application of GH and CAST markers in marker-assisted selection programs for Dagestan Mountain sheep and their crossbred populations.

To enhance the generalizability and reliability of these estimates, further validation across larger and more diverse multi-farm cohorts is recommended. These findings provide valuable insights for breeding programs aimed at improving meat productivity traits in sheep populations.

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