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Association of the growth hormone gene polymorphism with growth traits in Salsk sheep breed

Ivan F. Gorlov[a,b,*], Yuri A. Kolosov[b], Nadezhda V. Shirokova[a], Lyubov V. Getmantseva[c], Marina I. Slozhenkina[a,b], Natali I. Mosolova[a], Nekruz F. Bakoev[c], Maria A. Leonova[c], Anatoli Yu. Kolosov[c], Elena Yu. Zlobina[a,d]

[a] Volga Region Research Institute of Manufacture and Processing of Meat-and-Milk Production, 6, Rokossovskogo Street, Volgograd 400131, Russia
[b] Volgograd State Technical University, 28, Lenina avenue, Volgograd 400005, Russia
[c] Don State Agrarian University, 1, Krivoshlykov, Persianovsky 346493, Russia
[d] Volgograd State University, 100, University Avenue, Volgograd 400062, Russia

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ABSTRACT

The aim of the study was to identify the *GH/HaeIII* gene polymorphism and to determine its association with growth traits in Salsk sheep bred in the Southern region of the Russian Federation. The identification of the *GH* gene polymorphism was performed by the PCR-RFLP method using the endonuclease *HaeIII* for restriction of an amplified fragment. During the experiment, the AA, AB, and BB genotypes of the Salsk breed sheep were identified at a frequency of 57, 36, and 7%, respectively. The values of the weight at weaning, at the age of 9 months and the average daily gain of the ram lamb with the AB genotype exceeded the values of the these parameters of the ram lamb with the AA genotype by 0.92 kg, 10.67 kg, and 47.3 g, respectively. The carcass weight, the weight of meat, the slaughter weight, and the slaughter yield of the ram lamb with the AB/GH genotype were found to be more as compared with the parameters of the ram lamb with the AA/GH genotype by 4.97 kg, 1.83 kg, 4.83 kg, and 2.04%, respectively. The ram's AB genotype also caused a greater weight of heart and kidney by 75.21 and 75.44 g, respectively. Thus, the presence of a heterozygous AB genotype in Salsk sheep breed has a positive effect on the growth traits. The rams of the AB/GH genotype significantly exceeded the rams of the AA/GH genotype and were found to have the best meat productivity.

1. Introduction

Food shortage in the world encourages the search for the ways of increasing the production volume and preservation of the key food quality attributes. Meat is one of the main sources of protein, which is an essential element of the human diet. Sheep farming in developed countries has long been regarded as an industry – an important supplier of food for the population. Development of the ways of more efficient use of the gene pool of available sheep breeds in order to increase the level and the quality of meat and milk productivity, working out the methods for feed costs savings per unit of production, genetic monitoring, control of the selection process, and finding supplementary reserves that can improve the economic performance of the industry are the most important tasks of the sheep husbandry nowadays (Moradian et al., 2013; Vacca et al., 2013; Karagodina et al., 2014).

The Salsk sheep was bred during the period from 1930 to 1949 in the stud farm n.a. Budyonnov, the Rostov region of Russia. The breeders were V.P. Bashkatov, P.F. Karpov, M.I. Chumakov and others. The purpose was to create a breed, which would be well adaptive to the dry steppes and have high merino wool productivity. The Mazaevskij and Novokavkazskij Merinos were taken as a basis for the breed, improved by the in-breeding methods, and locally crossed with rams of the American Rambouillet breed. The result of the long-term breeding work, which mainly included a selection of the best breeding representatives, was the breed officially approved in 1950. The Salsk sheep in weight are characterized by a sound constitution. They are large and medium-sized, harmoniously built, have relatively long body, straight back, moderately

* Corresponding author at: Volga Region Research Institute of Manufacture and Processing of Meat-and-Milk Production, 400131, Rokossovskogo Street, 6, Volgograd, Russia.
E-mail addresses: niimmp@mail.ru (I.F. Gorlov), nadya.shirockowa@yandex.ru (L.V. Getmantseva), slozhenkina@mail.ru (M.I. Slozhenkina), nadya.shirockowa@yandex.ru (N.F. Bakoev), nadya.shirockowa@yandex.ru (M.A. Leonova), nadya.shirockowa@yandex.ru (A.Yu. Kolosov), elenazlobina2008@yandex.ru (E.Yu. Zlobina).

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wide chest. The characteristic feature is one large longitudinal fold and 1–2 transverse ones around the neck with an "apron". The live weight of adult rams is 80–100 kg, of ewes – 45–55 kg. True wool is crape-like staple, strong, 8–8.5 cm long. Closed fleece has a staple structure. The color of the coat is white. Wool grease is light. Animals have a high immunity and stamina. In terms of productivity, the Salsk breed is similar to the Soviet Merino. The carcass weight of rams is 30–33 kg with the slaughter yield of 50%. The carcass weight of calves is 14–16 kg with the slaughter yield – 43%. The sheep wool clip from a ram is 12–14 kg (to 17 kg), from a ewe – 5.5–6.5 kg (to 8 kg). The fineness of the wool from the ewes is mostly 20.6–20.3 microns, sometimes – 18.1–20.5, from the rams – 23.1–25 microns. The clean equivalent weight is 40–50%. The birth rate of the Salsk sheep is 120–140 lambs per 100 ewes. The main breed herd is in the Salsk rayon of the Rostov region. The Salsk sheep are perfectly adaptive to dry steppes of the region, long drives, sparse vegetation of pastures in dry summer. Unfortunately, for decades the selection work was mainly focused on improving the wool quality. Given the negative nature of the correlation relationship between the wool and meat productivities, the meatiness as a major commercial parameter appeared to be poorly worked out to date. Genomic selection in this case is regarded as a means to achieve the desired results as fast as possible and as a method for the most effective selection.

Currently, there is an increasing interest in technologies based on the DNA-markers, which are widely used in national breeding programs of some countries with developed husbandry and have a significant impact on improving the composition of the carcass, the meat quality, and efficiency of meat production (Valeh et al., 2009; Mikhailov and Getmantseva, 2013; Jia et al., 2014; Bahrami et al., 2015; Dettori et al., 2015; Gorlov et al., 2016). Genetic gene-associated markers (candidate genes) gain in popularity. Their protein product plays a significant role in formation and regulation of biochemical and physiological processes (Wallis et al., 2006; Bahrami et al., 2013; Proskura and Szewczuk, 2014; Kolosov et al., 2015). The gene itself must possess a variety of allelic variants (polymorphism) that are related with variability in the productivity level (Noor et al., 2001; Hajihosseinlo et al., 2013; Ahlawat et al., 2014).

One of the most promising candidate genes is the growth hormone gene (*GH*). The growth (somatotrophic) hormone has a broad spectrum of biological activity and affects all body cells. It enhances the biosynthesis of protein, the DNA, the RNA, and glycogen and promotes the mobilization of depot fat and the disintegration of higher fatty acids and glucose in the tissues. In addition to the activation of anabolic processes accompanied by an increase in body size and stimulation of skeletal growth, the somatotrophic hormone coordinates and controls the flow rate of metabolic processes (Wickramaratne et al., 2010; Gorlov et al., 2014; Malewa et al., 2014; Othman et al., 2015; Seevagan et al., 2015; Singh et al., 2015). The growth hormone is a protein with a molecular weight of about 22,000; its polypeptide chain consists of 191 amino acid residues. The gene polymorphism located in exon 3 may be determined by PCR-RFLP using the restriction endonuclease *HaeIII*. The research conducted for the study of polymorphism in different sheep breeds pointed out a statistically significant relation between the *GH/HaeIII* genotypes and the weight and growth traits (Palmer et al., 1998; Tohidi et al., 2013).

In this regard, the study is conducted in order to identify the *GH/HaeIII* gene polymorphism and to determine its association with growth traits in Salsk sheep bred in the Southern region of the Russian Federation.

Table 1
Characteristics of the primers used for PCR amplification.

Gene	Primer	Fragment length, bp
GH	5′-GGAGGCAGGAAGGGATGAA-3′ 5′-CCAAGGGAGGGAGAGACAGA-3′	934

Table 2
The alleles and genotypes frequencies for the *GH* gene in Salsk sheep breed.

Gene	Alleles, %		Genotypes, %		
	A	B	AA	AB	BB
GH/HaeIII	0.75	0.25	57	36	7

2. Materials and methods

The work described has been carried out in accordance with The EU Directive 2010/63/EU for animal experiments. The investigations were carried out on the Salsk sheep breed in the Southern Federal District, Russia.

The molecular-genetic analysis established the presence and the frequency of alleles and genotypes. The effect of the *GH* gene genotypes on the growth rate of the rams ($n = 50$) was investigated according to the following criteria: the weight at birth, weaning at 2 months and 9 months of age (kg); the average daily gain (in the period from 2 to 9 months of age) (g). The meat quality of the ram lamb was considered with respect to the results of the control slaughter at the age of 9 months on the following parameters: the slaughter weight (kg), the weight of meat (half-carcasses) (kg), the slaughter yield (%), the weight of internal organs (spleen, lungs, heart, liver, kidneys) (g). All the test animals were one year of birth with minimal difference in age. They were kept in the same conditions of feeding and daily regimen and serviced by the same staff.

To carry out the molecular-genetic studies, the tissue samples of 1 cm^2 were taken from the ear area of the animals ($n = 84$). DNA was isolated using a kit of reagents DIAtom™ DNA Prep 100 (LLC Research and Production Company Genlab, Russia). Analysis was performed by the PCR-RFLP. Characteristics of primers used for PCR amplification are presented in Table 1.

The following protocol was applied: predenaturation at 95 °C for 5 min and then 33 cycles of 95 °C for 45 s, 60 °C for 45 s, 72 °C for 45 s, the final synthesis performed at 72 °C for 10 min. Restriction of the amplified fragment was performed by endonuclease *HaeIII*. In the presence of 10 restriction sites were formed that corresponded to the A allele, in the presence of 11th – to the B allele. The size of the resulting restriction fragments were determined by electrophoresis in 4% agarose gel in the presence of ethidium bromide.

The presence and the frequency of alleles and genotypes were established according to the results of the molecular genetic analysis (Nei and Kumar, 2000). The allelic and genotypic frequencies, the heterozygosity observed (Ho) and expected (He), and the Hardy–Weinberg equilibrium test were calculated using PopGene 3.1 software. The frequency of genotypes was determined by the formula $p = n/N$, where p is the frequency of the genotype determination, n is the number of individuals with a specific genotype, and N is the number of individuals. The frequency of certain alleles was determined by the formula $PA = (2nAA + nAB)/2N$, $qB = (2nBB + nAB)/2N$, where PA is the frequency of allele A, qB is the frequency of allele B, and N is the total number of alleles. The expected results of the genotype frequencies in the studied population were calculated by the Hardy–Weinberg law.

The data on different variables, obtained from the experiment, were statistically analyzed by Statistica 10 package (StatSoft Inc.). The significance of differences between the indices was determined using the criteria of nonparametric statistics for the linked populations (differences with $P < 0.05$ were considered significant: $^{***}P < 0.001$; $^{**}P < 0.01$; $^{*}P < 0.05$; ns = not significant at $P > 0.05$). Student's t-test was applied for the statistical analysis (Johnson and Bhattacharyya, 2010).

3. Results and discussion

In the first phase, the molecular genetic study of the Salsk sheep identified the allelic variants of the *GH* gene (Fig. 1) and established the genotypes presented by the following fragments: 277-, 202-, 110-, 100-, 94-, 68-, 49-, 22-, 8-, and 4 bp were identified as the AA genotype; 256-, 202-, 110-, 100-, 94-, 68-, 49-, 22-, 21-, 8-, and 4 bp – as the BB genotype; 277-, 256-, 202-, 110-, 100-, 94-, 68-, 49-, 22-, 21-, 8-, and 4 – as the AB genotype. The frequency of the AA, AB, and BB genotypes was defined during the experiment in the ratio of 57, 36, and 7%, respectively. The A allele and the homozygous AA genotype had the highest frequency in sheep of Salsk breed (Table 2).