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Full Length Article

Growth Hormone *(GH)* Gene Polymorphism and Its Association with Meat Productivity in Two Rough Wool Sheep Breeds Grown in Russia's Dry Zone

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Abstract

The article considers the methods of efficient use of the gene pool of rough wool sheep breeds in order to improve the level and quality of meat production. The research material was Edilbay (n=100) and Kalmyk fat-tailed sheep (n=100) breeds. The influence of the polymorphism of the genotypes of the growth hormone gene on meat indices were established; and the desired genotypes for further breeding work to increase their specific weight in the population structure were determined. The *AB/GH* genotype associated with the best meat production in carriers of this genotype has been identified as desirable. The results of the control slaughter have indicated Kalmyk fat-tailed rams of the *AB/GH* genotype to be superior to their *AA/GH* and *BB/GH* genotypes peers in terms of their pre-slaughter weight. The conducted research indicated the feasibility of genetic markers to optimize breeding programs in flocks of rough wool sheep in order to increase the meat production level. The obtained data confirmed the need to investigate the DNA markers associated with the productive qualities of sheep to get greater efficiency of breeding and enhance profitability of the sheep industry. © 2021 Friends Science Publishers

Keywords: Growth hormone gene polymorphism; MAS; PCR-RFLP; Sheep breeding; Slaughter traits; SNP

Introduction

Currently, the Russian Federation has great potential both for increasing the sheep population and for the growth in production of all types of products in sheep industry (Lescheva and Ivolga 2015). On its territory there is large space of natural pastures that farmers can rationally use to meet the need of sheep for feed without considerable material costs; there are unique sheep breeds (Edilbay and Kalmyk fat-tailed) characterized by a high genetic potential of production and good ability to adapt to various climatic conditions; there is a substantial demand for organic meat products of sheep production both in Russia and abroad. Over the past 20–25 years, the consumption of mutton in the country has remained stable (1–1.2 kg/person per year), and a positive shift in the industry can only be expected when a steady consumer demand is formed (Fisinin 2017). In the early 2018, Russia imported 1.7 thousand tons, with the export of mutton amounting to 4.1 thousand tons against 41 tons for the same period last year. Iran was the main consumer of Russian lamb. This can be largely explained by the fact that in May, on the margins of the Astana Economic Forum, the EAEU countries and Iran signed a free trade agreement that fixed that the import duty on these products would not exceed 5%. Thus, taking into account the export of mutton, there was recorded a high positive dynamics of consumer demand among foreign trade partners. This indicates good prospects for sheep industry.

According to the Federal State Statistics Service, at the beginning of 2018, the sheep livestock in farms of all categories amounted to 23 million head; 146.2 thousand of them belonged to the Edilbay breed and 44.1 thousand to the Kalmyk fat-tailed breed. Having large areas of natural rangelands, the regions of the Southern Federal District are a

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traditional place of breeding sheep of unique breeds characterized by a high genetic potential of productivity and adapted to breeding in various climatic conditions. In the Southern Federal District, the sheep population numbers 6.3 mil. head; 56.5 thousand of them are of Edilbay breed, and 43.3 thousand of Kalmyk breed.

Each sheep breed is characterized by a unique gene pool and results from long, focused and hard work. Long-term intensive selection of breeds creates stable co-adaptive gene complexes that determine specific characteristics of a particular breed and the adaptive rate of populations (Rasali *et al.* 2006; Deniskova *et al.* 2018).

Sheep breeding has historically been an integral part of the national economy, especially in the North Caucasus and Southern Federal Districts, providing industry's needs for specific types of raw materials (wool, krimmer skin and sheepskins) and the population's needs for food (lamb and milk) (Zinovieva *et al.* 2015). In the recent past, the economy of sheep farming in our country was based mainly on wool production. Its share in the total cost of this industry production was 60–80%, and the selling price of 1 kg of wool was equivalent to 20 kg of mutton in live weight. At the same time, the ratio of prices for wool and lamb was 1:3 in the world market.

Lamb is one of the most valuable types of meat products and is in high demand in the world market (Buschulte *et al.* 2005). Resulting from the increasing economic importance of meat production, the rough wool sheep breeds – Edilbay and Kalmyk fat-tailed with their all-purpose production, especially meat production – have become important. The uniqueness of rough wool sheep breeds lies in their abilities and traits being not characteristic of animals in other production areas, *i.e.*, high resistance to infectious diseases, adaptability to rapid temperature changes and ability to overcome long distances during drifts to pastures.

Developing methods for more efficient use of the gene pool of rough wool sheep to enhance the quality of meat production, reduce feed costs per unit of production, genetic control and breeding management and find additional reserves that are able to improve the economic performance of the industry are the most important tasks at the present stage of sheep breeding development (Deniskova *et al.* 2016; Gorlov *et al.* 2018a; Bayram *et al.* 2019; Ekegbu *et al.* 2019). The growth hormone *(GH)* gene is considered as a marker of sheep meat production (Tahmoorespur *et al.* 2011). The GH is of great importance for regulating growth processes, cell proliferation and differentiation in all mammalian species. Somatotropin has a powerful anabolic and anti-catabolic effect, enhances protein synthesis, inhibits its breakdown and helps reduce subcutaneous fat deposition, increase fat burning and increase the ratio between muscle and adipose types of mass. The GH is a protein with a molecular weight of 22,000; its polypeptide chain consists of 191 amino acid residues (Gorlov *et al.* 2017).

The purpose of our investigation was to study the *GH* gene polymorphism that allows selection of rough wool sheep genetically predisposed to high meat production. Furthermore, the obtained data confirmed the need to study the DNA markers associated with the productive qualities of sheep to get greater efficiency of breeding and enhance profitability of the sheep industry.

Materials and Methods

Sample collection and genomic DNA isolation

The research material was sheep of Edilbay (n=100) and Kalmyk fat-tailed (n=100) breeds (males) of the herd in the Kirovsky breeding plant, the Yashkulsky rayon in the Republic of Kalmykia. For molecular genetic studies of sheep (n=200), tissue samples of 1 cm² were taken from the auricle. The DNA was isolated using Nexttec columns (Nexttec GmbH, Germany) in accordance with the manufacturer's recommendations.

PCR analysis and SNP genotyping

The data were analyzed by the method of PCR-RFLP. For amplification of a fragment of the GH gene 934 bps long, there were used oligonucleotide primers: 5'-GGAGGCAGGAAGGGATGAA-3' and 5'-CCAAGGGAGGGAGAGACAGA-3' (Gorlov *et al.* 2017). The composition of PCR mixture for amplification was following (in a final reaction volume of 25 μL): about 100–150 ng of isolated DNA (5 μL in average); 0.5 μL of each oligonucleotide primers (forward and reverse); 1 μL mixture of dNTPs; 5 μL of PCR buffer, and 0.3 μL of Taq DNA Polymerase (Tersus Plus PCR kit, Evrogen, Russia).

The amplification mode contained pre-denaturation at 95°C for 5 min. and then 33 cycles: 95°C for 45 s, 60°C for 45 s and 72°C for 45 s; final synthesis at 72°C for 10 min. The PCR was done on a thermocycler Tertsik, Russia. The amplified fragment was reduced by the endonuclease *HaeIII*. For the restriction analysis of the obtained PCR products in a final volume of 10 μL, there were mixed 6 μL of a PCR product, 2.5 μL of ddH2O, 0.5 μL of the *Hae III* endonuclease restriction enzyme (SibEnzyme-M, Russia) and 1 μL of buffer for the enzyme. The hydrolysis was carried out at a temperature of 65 0C for 1 h in a thermostat TT-2 Termit (NPO DNA-Tekhnologiya LLC, Russia). The sizes of the fragments were determined in comparison with the molecular weight marker M100 (Izogen, Russia, the length of the fragments 100 to 1000 bp) supplied with 1 mL of 6xDNA dye on 4% agarose gel (Helicon, Russia) and stained with ethidium bromide (Helicon, Russia). The resulting restriction fragments were visualized in ultraviolet light. The presence of 10 restriction sites corresponded to allele A, the presence of 11 sites to allele B. The size of the restriction fragments obtained was determined by electrophoresis in a 4% agarose gel with ethidium bromide. The molecular genetic analysis established the presence and frequency of alleles and genotypes.

The presence and the frequency of alleles and genotypes were established similarly Gorlov *et al.* (2017). The allelic and genotypic frequencies, the heterozygosity observed (Ho) and expected (He), and the Hardy-Weinberg equilibrium tests were calculated by Pop Gene 3.1 software. The research allowed us to solve the problem of assessing the state of the sheep populations under study in terms of the statistical significance of differences in the values of the heterozygosity observed and expected. The frequency of heterozygotes is an important indicator, since each heterozygous individual carry different alleles and thus indicates the presence of variability.

Slaughter traits

The control slaughter of rams aged 4 months helped establish the meat qualities (in accordance with the requirements of the GOST 31777-2012 "Sheep and goats for slaughtering. Mutton, lambs and goats in carcasses specifications") with respect to parameters, *i.e*., the pre-slaughter weight (kg), weight of fresh carcass (kg), weight of chilled carcass (kg), slaughter weight (kg), and slaughter yield with fat-tail (%). All animals studied (100 animals in each group) were the same year of birth with minimal differences in age, kept in the same feeding conditions and daily routine and served by the same employees.

Statistical analysis

The data on different obtained variables were statistically analyzed by Statistica 10 package (StatSoft Inc.). The significance of differences between the indices was determined by the criteria of nonparametric statistics for linked populations (differences with $P < 0.05$ were considered significant; NS = not significant at $P < 0.95$). Student's t-test was applied for the statistical analysis (Johnson and Bhattacharyya 2010).

Results

Molecular genetic studies of biological samples obtained from sheep of Kalmyk fat-tailed breed (Fig. 1A) allowed establishing a *GH* polymorphism pattern caused by the ratio between alleles *A* and *B*. The allele *A* and *AA* genotype had the highest frequency in the sheep group under study. The frequencies of the AA, AB and BB genotypes were set at a ratio of 52.0, 35.0 and 13.0%, respectively (Fig. 2). The average value of the heterozygosity observed (Ho) in Kalmyk fat-tailed breed was found 0.350 and expected (He) was higher – 0.574. The research studies of the biomaterial obtained from Edilbay sheep (Fig. 1B) found, that the *GH* gene polymorphism represented by *A* and *B* alleles had a slightly different structure. In the population studied, the highest frequency was also characteristic of the allele *A* and homozygous *AA* genotype (Fig. 2). However, the picture of the frequency of the *AA*, *AB* and *BB* genotypes identified in

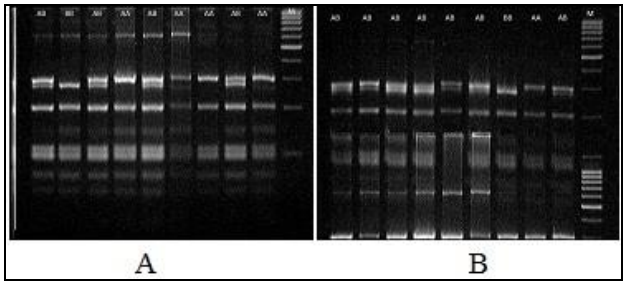

Fig. 1: Electropherograms of the PCR-RFLP result of the *GH (HaeIII)* in 4% agarose gel (M is DNA marker – 100 bp): (**A**): Kalmyk fat-tailed sheep breed; (**B**): Edilbay fat-tailed sheep breed

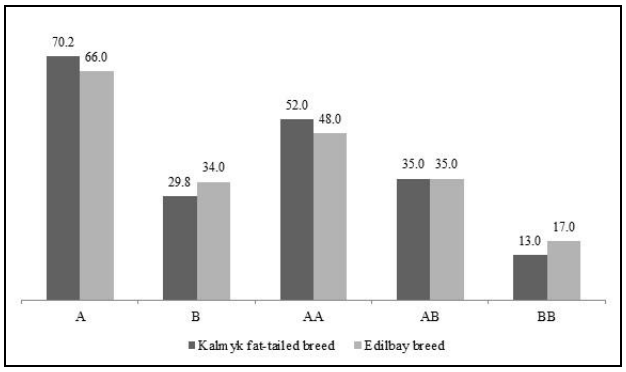

Fig. 2: The frequency of alleles and genotypes of the *GH* gene in Kalmyk fat-tailed breed and Edilbay breed

this group looked different than in Kalmyk fat-tailed group, namely: 48.0, 35.0 and 17.0%, respectively. So, the proportion of *AA* homozygotes was noted to have slightly decreased, and the *BB* homozygotes proportion was registered to have increased. The proportion of heterozygous *AB* genotype remained virtually unchanged. The average value of the heterozygosity observed (Ho) in Edilbay breed was found 0.350 and expected (He) was higher – 0.448.

The values of the heterozygosity observed and expected in the studied populations of sheep of Kalmyk fat tailed and Edilbay breeds are presented in Table 1. In our work, in all cases, there was found no statistical significance of differences in the values of the heterozygosity observed and expected. The distributions of heterozygous genotypes observed reliably corresponded to those expected by Hardy-Weinberg's equilibrium law; according to the χ^2 value obtained, the populations under study were in equilibrium.

Further studies on the relationship between the allelic *GH* gene variants and indicators of rough wool sheep meat production showed that the best meat productivity belonged to the *AB/GH* rams that significantly exceeded their *AA/GH* analogs with respect to almost all traits analyzed (Table 2). The analysis of the control slaughter data showed that Kalmyk fat-tailed sheep of the *AB/GH* genotype surpassed their *AA/GH* and *BB/GH* genotypes peers in terms of the pre-slaughter weight by 3.7 ($P < 0.001$) and 2.6 kg ($P <$

Table 1: Observed and expected heterozygosity of the growth hormone gene in sheep populations

Breed	Number of animals (n)	Heterozygosity observed (H_O)	Heterozygosity expected (H_E)	χ^2
Kalmyk fat tailed breed	100	0.350	0.574	3.04
Edilbay breed	100	0.350	0.448	5.09

Table 2: Slaughter traits of rams of different *GH* genotypes (M ± SE)

Genotype	Pre-slaughter live weight (kg)	Carcass fresh weight (kg)	Carcass cooled weight (kg)	Slaughter weight (kg)	Meat yield (per kg bones)	Slaughter yield with fat-tail (%)
Kalmyk fat-tailed breed (n=100)						
AA (n=52)	38.0 ± 0.79^{a0}	16.4 ± 0.81^{b0}	16.0 ± 0.73^{bc}	19.3 ± 0.72^{c0}	$3.47 \pm 0.03^{a***}$	50.7
AB (n=35)	$41.7 \pm 0.72^{***}$	$18.9 \pm 0.40^{***}$	$18.4 \pm 0.54^{***}$	$21.5 \pm 0.49^{***}$	$3.56 \pm 0.01^{***}$	51.6
BB (n=13)	39.1 ± 0.45^{b0}	16.9 ± 0.64^{bc}	16.3 ± 0.58^{bc}	$20.1 \pm 0.31^{c***}$	$3.16 \pm 0.04^{a***}$	51.1
Edilbay breed (n=100)						
AA(n=48)	36.8 ± 0.46	14.9 ± 0.34	14.3 ± 0.38	18.3 ± 0.42	2.93 ± 0.04	48.9
AB (n=35)	38.4 ± 0.49^{C}	16.2 ± 0.36^{B}	15.6 ± 0.33^{C}	19.5 ± 0.28^{C}	3.03 ± 0.02^{c}	50.7
BB (n=17)	37.4 ± 1.22^{NS}	15.3 ± 0.12^{NS}	14.9 ± 0.10^{NS}	18.6 ± 0.21^{NS}	3.02 ± 0.01^{c}	49.7

Note: a = $P < 0.001$, b = $P < 0.01$, c = $P < 0.05$, ns = not significant at P<0.95 compared with data on the AB-genotype in Kalmyk fat-tailed breed group; A = $P < 0.001$, B = $P < 0.01$, C = $P < 0.05$, NS = not significant at $P < 0.95$ compared with data on the AA-genotype in Edilbay breed group; *** = $P < 0.001$, ** = $P < 0.01$, * = $P < 0.05$, ^{0} = not significant at $P < 0.95$ compared with data on the similar genotype in the different breeds group

0.01). The slaughter weight and slaughter yield of the *AB/GH* genotype rams also exceeded these parameters in comparison with the *AA/GH* genotype rams by 2.2 kg ($P < 0.05$) and 0.9%, respectively. A similar pattern was observed when comparing the heterozygous *AB/GH* and homozygous *BB/GH* genotypes of rams. The slaughter weight and slaughter yield of the *AB/GH* genotype rams were more by 1.2 kg ($P < 0.05$) and 0.5%, respectively.

By all parameters of the control slaughter, Edilbay rams of the *AB/GH* genotype exceeded their *AA/GH* and *BB/GH* genotypes peers. So, in Edilbay breed, the pre-slaughter live weight of the *AB/GH* genotype rams was higher than that of the *AA/GH* and *BB/GH* genotypes rams by 1.6 ($P < 0.05$) and 1.0 kg (NS), and the slaughter yield by 1.8 and 1%, respectively.

Discussion

In recent years, a number of reports have appeared that noted the relationship between the different gene polymorphisms and the meat production of sheep bred in Russia (Gorlov *et al.* 2016; Trukhachev *et al.* 2016, 2017; Gorlov *et al.* 2018b). The purpose of these studies was to enhance the level and quality of meat production, as well as reduce the cost of feed per unit of production in Russian sheep breeding. The *GH* gene studies by Gorlov *et al.* (2017) on Salsk sheep of Russian breeding established all three *AA*, *AB* and *BB* genotypes' frequencies of 57, 36 and 7%, respectively. The frequency of the allele *B* was 0.25 and allele*A*0.75. In the studies conducted, the heterozygous genotype of Salsk sheep was associated with the best average daily gains in the period from 2nd to 9th months.

Similar results were obtained by Kolosov *et al.* (2015), who proved the positive effect of the heterozygous *AB/GH* genotype on the average daily gains and meat production of Merino sheep breeds.

Most researchers believe that the issue of increasing the level and quality of meat production can be solved by genetic methods. In this regard, the GH gene looks the most promising. So, in the studies conducted by Hajihosseinlo *et al.* (2013) on Makooei sheep, the best indices of meat production were associated with the *AB* genotype.

During previously conducted studies to identify the relationship between the polymorphism and the growth and weight characteristics of sheep of various breeds, similar results were obtained. According to the research by Palmer *et al.* (1998) on crossbred sheep from crossing Dorset and Coopworth breeds, it was found that the *AB* genotype sheep gained more than the *AA* genotype sheep by 123 g per day or 18%. Thus, the sheep *GH* gene polymorphism can be used as a marker for weight gain and higher meat production.

Conclusion

The results of the *GH* gene polymorphism in sheep of two rough wool breeds grown in the arid zone of Russia were obtained for the first time; and significant associations between the *GH* gene genotypes and sheep meat productivity were revealed. The influence of the polymorphism of the genotypes of the growth hormone gene on meat indices were established; and the desired genotypes for further breeding work to increase their specific weight in the population structure were determined. The conducted research indicated the feasibility of genetic markers to optimize breeding programs in flocks of rough wool sheep in order to increase the meat production level.

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Author Contributions

Conceptualization: IFG, YuAK, NVSh; Data curation: AYuK, NIM, AKN; Formal analysis: MIS, AKN; Funding acquisition: EYuA, DVN; Investigation: NVSh, AYuK; Methodology: IFG, YuAK; Resources: MIS, AKN; Supervision: IFG, YuAK; Writing-original draft: EYuA.

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