

Allele Fund Characterization Of Edilbay Sheep By Dna-Markers Polymorphism

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Abstract

Due to advanced development of applied scientific research in the field of agriculture, creating methods of genomic selection based on DNA technology determines the relevance of the present research. Studying polymorphic systems of blood proteins of fat-tailed sheep, the authors determined the degree of differentiation of the genetic structure of Edilbay sheep by alleles and their

correlation with productive qualities. The analyses revealed that the GH-2 gene influences the synthesis of development hormone and on the whole organism of experimental sheep in indirect ways, including the secretion of insulin-like growth factor IGF-1. Due to their role in regulating cell proliferation and muscle tissue development through the stimulating effect of testosterone, the GH-2 and IGF-1 genes are considered as candidate markers for indicators of sheep meat productivity.

Keywords: genomic selection, fat-tailed sheep, Edilbay breed, live mass, growth rate, indicators of control slaughter, meat ratio, DNA-analysis, marker genes.

Introduction

Increasing precocity of farm animals is a topical issue not only in the agricultural sector but also in biological science and livestock breeding in general. It is inextricably linked with minimizing material, labor and financial costs of livestock production, which fully complies with the modern market economy requirements.

Specific climatic conditions and vast pastures of Kazakhstan, combined with social significance, contributed to the development of fat-tailed sheep breeding of the specialized meat production line (Smagulov, 2017). The most valuable features of these native sheep are unsurpassed endurance, high vitality and early maturity of young animals, and the minimum need for feed and veterinary protection. Moreover, they are considered part of the culture of a whole nation, a link in the evolution of a species, and reflect the patterns of the geographical gene pool formation (Sadykulov, 2000).

At present, fat-tailed sheep can become an independent source for lamb meat production both in the regions with natural pastures and in farms practicing stall-feeding of young animals.

Dry-steppe and semi-desert zones of the Western region of Kazakhstan have great potential for increasing both the number of sheep and production volumes. The total land fund of the region is 15 133.9 thousand ha, of which 13 907.3 thousand ha are agricultural land. These territories have significant arrays of pasture resources of over 10 million ha which can be rationally used without significant material costs when grazing sheep (Traisov, Ukbaev, Smagulov 2016).

The number of sheep in the West Kazakhstan region as of January 1, 2020 is 1,390,615 heads, including ewes (more than 651.5 thousand heads), and the share of the breeding stock is about 50.0 thousand heads, 15% of which are semi-fine-wool sheep of the Akzhaik breed with crossbred wool and 85% are coarse-wool sheep, mainly of the Edilbay breed (Official MARK website).

The Edilbay breed, which is the national pride of the Kazakh people, is of great interest among fat-tailed breeds, as it ranks high among all the bred sheep worldwide in quantitative and qualitative indicators of meat productivity. The breed has unique economically useful traits: stable adaptability in the zone of dry steppes, semi-deserts and deserts, high early maturity of young animals at the level of the best genotypes, only slightly inferior in terms of deposition and size of tail fat to the Hisor breed, possessing at the same time relatively better meat forms.

The live weight of sheep-producers is 100-120 kg, of ewes – 65-75 kg. Being very precocious, at the age of 4-4.5 months Edilbay lambs and ewes weigh 38-45 and 35-40 kg, respectively. The wool of Edilbay sheep is of medium and higher than average quality when compared with similar breeds; a ram gives 3.0-3.5 kg of wool, an adult ewe – 2.0-2.5 kg. The ewes produce a large amount of milk, and this favors the good development of lambs in the suction period (270-300 g); fecundity, depending on the feeding conditions of the year, ranges from 100 to 120%.

The main livestock of Edilbay sheep is concentrated in the following breeding farms: Birlik, Edilbay and Kamkor of West Kazakhstan Region, Suyndik, Kurmangazy and Makash of Atyrau Region, Islambek, Nur-Asyl, and Rakym of Aktobe Region. These farms are the main reproducers of the pedigree Edilbay genotypes used to improve the productive qualities of fat-tailed sheep in all regions of Kazakhstan.

The rational use of specific combinations of the genetic potential of sheep meat and the creation of promising populations with high productivity and consolidated heredity, combined with valuable biological properties, are a unique opportunity that must be used to quickly increase the profitability of this industry. Such gene pool will allow solving both tactical and strategic tasks in breeding sheep in Kazakhstan (Smagulov et al., 2014).

To achieve high sheep productivity indicators, it is necessary to use all the reserves available in sheep breeding, and transfer the industry to intensive technology. Along with improving the feed base and the keeping technology, the realization of potential breeds is of great importance in increasing production.

Further improvement in sheep productivity is not possible without modern advances in agricultural genetics. In numerous countries, new approaches based on the use of genetic markers of breeding traits are widely employed to create breeds, types, and lines of animals. Marking at the genotype level, in addition to traditional classical breeding methods, can significantly increase the efficiency of breeding and achieve the desired result within several generations (Kostyunina, 2008; Zinovieva, 2010).

When creating methods of test systems for multilocus genotyping of farm animals, different approaches are currently used, in particular, DNA biochips for detecting mononucleotide substitutions - single nucleotide polymorphisms (SNPs). An alternative to the SNP detection method is the development of simultaneous genotyping of a number of loci of anonymous DNA fragments flanked by inverted repeats of microsatellites. Such multilocus spectra allow creating genomes 'bar coding' on the basis of anonymous DNA fragments of different lengths, which makes it possible to reliably assess whether the animals or the products obtained from them belong to a certain species, breed, and inbreed group (Gui et al., 2016; Toghiani et al., 2017; Meseret et al., 2020).

Studying the involvement of different genetic systems in forming old and new breeds can significantly contribute to understanding the genetic mechanisms of artificial selection; this will allow accelerating and shortening the time of breeding, and finding qualitatively new approaches for selecting parental forms to obtain heterotic progeny (Legarra et al., 2014; Novikov, Semak, Khrunova, 2016).

Markers based on the hybridization reaction or on a PCR stage which detect DNA polymorphism are currently used in various fields of biology, including study and conservation of genetic diversity, identification of individuals, phylogenetics, mapping of useful traits of quality and resistance to stress factors in the breeding process, and in biotechnology (Tan et al., 2017; Lemos Serrote et al., 2020; Kumar et al., 2020).

Correct interpretation of the of genotyping results imply taking into account the fact that using any type of marker is associated with a number of genotyping errors, the main ones being the loss of large alleles, the 'zero' allele, the 'stutter' allele, due to the characteristics of Taq-polymerase and the non-homologous amplification (homoplasia). Minimizing errors is achieved by evaluating the capabilities of a certain type and by screening markers, by optimizing experimental methods, making proper use of controls and replicates, and by developing statistical approaches to detect errors. The trade-off between rejecting loci that generate errors and increasing the potential of the remaining loci to amplify the genetic signal may be different in different studies; however, this signal is not to be lost for the sake of an 'acceptable' error level, and researchers are to develop empirical approaches to achieve the desired result.

Therefore, research aimed at determining a certain genetic identification, accurate control of the origin and markers of animal productivity is an important step for the development of modern animal husbandry. Appropriate genetic passports help identify the polymorphic loci panel and conduct genetic monitoring of farm animals.

Materials and Methods

The objects of research were purebred Edilbay sheep of different sex and age groups, bred in the Edilbay farm of the Akzhaik district of the West Kazakhstan region. Standard methods of organizing zootechnical experiments were used; all the rams used in the experiment belong to the elite class, the ewes being not lower than I class, and the control groups were formed by the principle of analogue pairs.

For screening, 300 sheep were selected. In the DNA study, samples of the biological material of sheep (blood) were taken in test tubes and contained EDTA anticoagulant. Using the Extran-2 reagent kit (Sintol, Russia) and Nexttec columns (Nexttec Biotechnologie GmbH, Germany), the genomic DNA of the tissue was isolated in accordance with the manufacturer's protocols.

The purity and integrity of genomic DNA was assessed on electrophoresis gel with a Nano Drop-8000 spectrophotometer. The concentration of DNA preparations was determined on Qubit (Invitrogen).

The analysis of polymorphism in the genes of the development hormone GH-2 and of the insulin-like growth factor IGF-1 was carried out using test systems that were specifically designed to perform research in the laboratory of molecular fundamentals of selection of All-Russian Research Institute of Animal Husbandry named after L.K. Ernst. Identification of single nucleotide substitutions in fragments of the GH-2 gene was carried out on the basis of PSQ96MA pyrosequencing (Pyrosequencing, Sweden) using

PSQ96MA SNP Software v. 2.0. IGF-1 gene polymorphism detection test systems were based on PCR-RFLP analysis.

Results and Discussion

The intensity of growth and development, as well as the level and quality of meat productivity of sheep, are the most economically useful signs. Identification of the molecular genetic mechanisms underlying the formation of these properties is of particular importance in terms of the growing market demand for high-quality sheep products.

In this regard, there is need for research aimed at studying the role of marker assessment of sheep using DNA analysis. Of particular importance among the polygenic systems of the body are blood proteins, such as genes for development hormone (GH), insulin-like growth factor (IGF), myostatin (MSTN) and fatty acid binding protein (FABP) (Sulimova, 2004; Leonova et al., 2013).

The search for allelic variants in candidate genes associated with breeding traits allows for early selection of sheep and reveals the genetic potential more fully, which is an urgent area of breeding (Ozerov et al., 2003).

As part of the study, testing was performed on the GH-2 and IGF-1 genes. These genes are promising candidate genes associated with economically useful traits of sheep (muscle tissue volume, growth rate, tenderness of meat, carcass variety, fat deposition in tissues, etc.).

Development hormone is a protein consisting of a single polypeptide chain, which is synthesized and secreted by anterior pituitary eosinophils in vertebrates. GH can speed up metabolism and promote growth of many organs and tissues, especially bone, muscle and visceral.

A study of the genetic structure of Edilbay sheep according to the polymorphism of the GH-2 gene showed the presence of genotypes AA, AG and GG (Table 1).

The highest frequency of occurrence was observed for the G allele (0.6473), which was found in 147 (49.0%) animals in the homozygous state and in 128 (42.7%) animals in the heterozygous state. The frequency of occurrence of allele A and genotype AA was 0.3527 and 8.3%, respectively.

In recent years, along with hormones, insulin-like growth factor was used as a DNA marker of meatiness in farm animals. The IGF-1 gene is involved in the regulation of such significant physiological processes as reproduction and development of the fetus (embryogenesis, histogenesis, morphogenesis and fetogenesis). It is assumed that IGF-1 is associated with the size of tails, amount of hair, amount of seed from rams, stable lactation in ewes and the size of the offspring (Table 2).

According to the authors' data, the CC genotype was predominant in the IGF-1 gene in the studied sheep population (70.3%). The frequency of occurrence of the C allele was significantly higher than the of T alleles: 0.7652 and 0.2348, respectively. Homozygous TT genotype was identified as the rarest – 6.7%.

The experiments revealed the frequency of occurrence of genotypes in the two studied genes associated with the growth rate and meat qualities of sheep. In the studied population, the GH-2 and IGF-1 genes are not monomorphic but polymorphic.

A comparative genomic analysis of animal DNA reveals some patterns in the distribution of the genotype in the breed aspect (Peter et al., 2007; Hayes et al., 2011; Gorlov et al., 2017; Ma et al., 2018; Getachewa et al., 2020). This direction is most promising for zootechnical practice in Kazakhstan, as it provides very broad opportunities for identification, certification and systematization of domestic breed resources. With only 15 types of fat-tailed sheep in the world, seven were bred in Kazakhstan by breeders and by selection scientists: Kazakh coarse-wool, Edilbay, Saryark, Degeress, Ordabasy, Kazakh semi-coarse-wool and Atyrau. This confirms the validity of the origin, formation and development of the ancestral homeland of the fat-tailed sheep breeding – dry-steppe and semi-desert natural zones of Kazakhstan.

When studying the influence of the abovementioned genes on live weight indicators of fat-tailed sheep, it was found that carriers of the heterozygous type AG of the GH-2 gene and the homozygous type CC of the IGF-1 gene grew more intensively compared to other genotypes.

Growth and development hormones have certain meanings on the regulation of some physiological body processes occurring in all types of mammals, and in particular, cell proliferation and differentiation. According to the results of genotyping, the meat qualities of the formed groups of sheep were estimated by indicators of live weight and average daily gain during the milky period of postnatal ontogenesis, as well as by carcass weight and slaughter yield during control slaughter at the age of 4-4.5 months, i.e. at the time of separating the offspring from the ewes (Table 3).

Analysis of the polymorphism of the GH-2 gene in Edilbay sheep showed some effect on body weight and growth energy of lambs. It should be noted the superiority of Sheep of genotype AG (45.8 kg at 305.3 g) which have higher live weight (by 2.1 kg or 4.8%) and average daily growth (26.9 g or 9.7%) are superior to their peers of homozygous genotypes AA (3.5 kg or 8.3%) and GG (23.6 g or 8.4%).

According to the IGF-1 gene, the most significant effect on live weight and daily average growth was detected among the SS genotype carriers. They exceeded their CT genotype peers by 2.5 kg or 5.6% and 14.2 g or 4.8%, and their TT peers by 4.1 kg or 9.6% and 25.4 g or 8.9%, respectively.

When assessing the rams by slaughter indicators, depending on the genotypes of the studied genes, it was found that groups with different polymorphic variants GH-2 and IGF-1 differ in the yield of internal fat and tail fat, as well as in the slaughter mass.

In the group of rams with the genotype AG of the GH-2 gene, the carcass weight was 22.9 kg with the slaughter yield of 50.0%. These indicators are higher than with the AA genotype (1.3 kg and 0.5%) and with the GG genotype (2.3 kg and 1.3%).

In rams with the genotype CC of the IGF-1 gene, the carcass weight reached 23.5 kg at 49.9% slaughter yield, which in comparison with the analogue groups (CT and TT) was also 1.6 and 2.7 kg more at 0.7 and 1.5%, respectively.

Notably, animals with the above genotypes had rather good indicators of live weight not only at the studied age but also throughout all the analyzed periods. Their accumulated mass during the suction period ranges from 42.3-47.0 kg (44.4 kg on average), which exceeds the III threshold of confidence on Student's t-test ($P > 0.999$). Admitting statistical errors (m_x) in the calculations using Past v. 2.17 s, its level would be only 727 g. To more fully realize the genetic potential of the Edilbay breed by improving the quality of breeding while minimizing the impact of the feeding condition and content technology, the possibilities of increasing this trait by ± 1.8 kg were determined; this is confirmed by the coefficient of phenotypic variability (C_v) – 12.5%.

In general, the live weight of young fat-tailed sheep (aged 4-4.5 months) was in full compliance with the requirements of the pedigree standard for the Edilbay breed. The body mass of sheep for the experiment exceeded the minimum threshold established for the elite class by 4.3 kg or 10.7%.

When analyzing the polymorphism of the GH-2 and IGF-1 genes, associated connections of different genetic variants with meat quality indicators were not revealed.

Conclusion

Identifying loci of quantitative traits of a polygenic nature and candidate genes associated with the properties of early maturity and meat productivity of Edilbay sheep is a prerequisite for the development of marker selection programs. The authors analyzed associations of genotypes of DNA markers of development hormone and of insulin-like growth factor. Assessment of the dynamics of live weight of rams depending on genotypes showed that individuals with AG of the GH-2 gene exceeded their AA and GG peers by 4.8 and 8.3%. According to the IGF-1 gene, rams with the CC genotype also had an advantage in this parameter over CT and TT by 5.6 and 9.6%, respectively.

The performed studies established a certain effect of the polymorphism of the above genes on the growth rate and meat quality of Edilbay sheep. The degree of correlation ($r = 0.7$) on the average daily gain of rams during the milk period of postembryonic ontogenesis and on the carcass weight was especially noticeable, which is of great importance for breeding with this population of sheep according to these characteristics.

Conflict of interest

The authors declare no conflict of interest.

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Tables

Table 1. Frequency of alleles and genotypes of GH-2 in the population

Parameters	Heads	Frequency				
		Genotypes			Alleles	
GH-2		AA	AG	GG	A	G
Study sample of sheep	300	25	128	147	0.3527	0.6473
	%	8.3	42.7	49.0		

Table 2. Frequency of alleles and genotypes of IGF-1 in the population

Parameters	Heads	Frequency				
		Genotypes			Alleles	
IGF-1		CC	CT	TT	T	C
Study sample of sheep	300	211	69	20	0.2348	0.7652
	%	70.3	23.0	6.7		

Table 3. Productive meat qualities of sheep of different genotypes

Genes	Genotypes	%	Live weight, kg	Average daily growth, g	Carcass weight, kg	Slaughter yield, %
GH-2	AA	8.3	43.7±0.21	278.4	21.6±0.05	49.5
	AG	42.7	45.8±0.34	305.3	22.9±0.02	50.0

	GG	49.0	42.3±0.36	281.7	20.6±0.14	48.7
IGF-1	CC	70.3	47.0±0.18	310.2	23.5±0.11	49.9
	CT	23.0	44.5±0.22	296.0	21.9±0.06	49.2
	TT	6.7	42.9±0.31	284.8	20.8±0.09	48.4