



**Full Length Article**

## Comparative Study of Two Sheep Breeds Grown in Russia: The *FABP4* Gene Polymorphism and Fat Deposition

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### Abstract

The laws of adipose tissue deposition and associative relationship between the *FABP4* gene polymorphism and coarse wool sheep breeds as a genetic marker for the meat production quality have been studied. Methods: The research material was the Edilbay (n=500) and Kalmyk fat-tailed (n=500) sheep breeds. The *FABP4* gene study was performed using polymerase chain reaction (PCR) with allele-specific primers. The PCR performance was detected by the gel electrophoresis. The alleles were identified by pyrosequencing. Results: The *FABP4* gene studies conducted on the sheep population of these breeds on the Kirovsky breeding plant in the Yashkul'sky rayon, the Republic of Kalmykia (Russian Federation) have determined only the homozygous form of the gene construct. The youngsters of Kalmyk fat-tailed breed were superior in terms of the weight of intramuscular and internal fat. The weight of their intramuscular fat was by 140 g more and of internal one by 40 g, or by 27.5 and 33.3%, respectively. Conclusion: The heterogeneity of Kalmyk fat-tailed sheep and, especially, Edilbay breed can be expected in Russia due to the diversity of breed resources used to create the Russian population of the Edilbay breed, as well as develop new approaches in breeding one of the most popular Russian sheep breeds widespread throughout the country. © 2020 Friends Science Publishers

**Keywords:** *FABP4*; Fat distribution; Pyrosequencing; Sheep farming; Single nucleotide polymorphism

### Introduction

The uniqueness of coarse wool sheep breeds lies in the fact that they possess traits that are not typical for animals of other production strains, *i.e.*, high growth rate at the initial stage of postembryonic development, resistance to infectious diseases, adaptability to rapid temperature changes and the ability to cover long distances in transhumance. The selection of these sheep is required not to lose their positive traits and, at the same time, increase the qualitative and quantitative productive characteristics. In the modern world, there is a growing need for exploring the early stage forecasting the qualities of meat obtained from sheep of various origins and utility strains as raw material for processing. The influence of such factors as breed, sex,

age and conditions of feeding and housing of animals, having a considerable impact on the development of meat production and meat quality, are studied quite fully. At the same time, there are biological properties associated with meat production. Against the background of the emergence of new assessment techniques, the study of the properties is of scientific and practical interest (Gerbens *et al.* 1999; Ardici *et al.* 2017).

Determining the genotype in its phenotypic manifestation continues to be an urgent task in the selection of farm animals (Ballester *et al.* 2017; Goszczynski *et al.* 2017; Ardici *et al.* 2019). Most of the economic traits are characterized by the polygenic nature of inheritance, which creates difficulties in carrying out the selection work on improving the traits selected (Barillet *et al.* 2005).

Modern market conditions considerably increase the level of quality requirements for products manufactured in the meat industry (Damon *et al.* 2006). So, it is increasingly required not only to enhance the live weight of the animal, but also improve the quality of the products obtained, that is, change the physicochemical properties of meat in the strain desired (Gorlov *et al.* 2017, 2018). Increasing the tenderness of meat due to traditional breeding techniques and methods is a difficult task, because the quality of meat is controlled by a number of genes, and their influence is sometimes multidirectional (Arsienko and Gladyr 2002; Gorlov *et al.* 2016).

Intramuscular fat content or marbling improves the tenderness of meat by reducing the density and strength of connective tissue, as well as a beneficial effect on taste and juiciness. Therefore, genes involved in the fatty acid metabolism are usually considered as potential candidate genes that affect the tenderness of meat (Adida and Spener 2006).

Gene mutations that affect muscularity (fleshiness), that is, muscle thickening in accordance with the parameters of the skeleton, as a rule, change the trajectory of the development program described, changing the number and composition of muscle fibers, as well as the nature of their trophism. There were identified many mutations that increase muscularity and influence the muscle development program described due to new mechanisms of action (Gerbens *et al.* 2001).

Considering the breeding work based on genetic markers as a way to increase the production of meat sheep breeding, one should not forget about the already known gene mutations associated with the deficiencies or diseases of animals, since a sick animal is not able to implement even the most promising genetic fleshiness program.

Fatty acid binding protein (*FABP*) genes are considered as possible markers of meat production and meat quality (Huang *et al.* 2006; Zhang *et al.* 2013). The *H-FABP* is one of the genes in this family and of great interest as a gene candidate for the content of intramuscular fat that is the most important parameter, determining the meat quality, and as a possible genetic marker for a decrease of the fat content in a sheep carcass (Barillet *et al.* 2005; Damon *et al.* 2006; Aviles *et al.* 2013; Lu *et al.* 2019).

The *FABP4* gene belongs to the group of so-called multigens and plays an important role in the lipid beta-oxidation (Adida and Spener 2006). It causes the production of protein that is responsible for the transport and intracellular metabolism of long-chain fatty acids (Kucharski and Kaczor 2017; Liu *et al.* 2018; Migdal *et al.* 2018).

Due to the lack of data on the polymorphism of this gene and its association with the character of fat deposition in the body of coarse wool sheep breeds in arid areas of the Russian Federation, the research team set a goal to study this issue on a representative sample of livestock (1000 heads of sheep).

## Materials and Methods

### Sample collection and genomic DNA isolation

The research material was the sheep of Edilbay (n=500) and Kalmyk fat-tailed (n=500) sheep breeds of the herd on the Kirovsky breeding plant in the Yashkulsky rayon, the Republic of Kalmykia (Russian Federation). Experiments were performed in accordance with the Guide for the care and use of laboratory animals (Committee for the Update of the Guide for the care and use of laboratory animals 2011). And the use of experimental animals completely observed the local animal welfare laws and policies. The current study is compliance with ethical standards. The authors declare that animal tissue samples were collected by trained personnel under strict veterinary rules. Sampling was performed in accordance with the ethical guidelines of the L.K. Ernst Federal Science Center for Animal Husbandry. The authors confirm that the sheep owner (Badma Esinovich Garyaev, Director General of Plemzavod "Kirovskiy") provided written consent for the use of his animals in this study.

### PCR analysis and pyrosequencing

The *FABP4* gene study was performed using polymerase chain reaction (PCR) with allele-specific primers. The DNA was isolated using the Nexttec columns (Nexttec GmbH, Germany) in accordance with the manufacturer's recommendations. Special oligonucleotide primers are presented in Table 1.

The PCR was set up at the following temperature and time modes: cycle I at 95°C for 5 min, 35 cycles sequentially at 95°C for 45 s, 63°C for 45 s, 72°C for 45 s and final elongation at 72°C for 7 min.

The PCR performance was detected by the gel electrophoresis. Five  $\mu$ L of amplification were introduced into 1.5% agarose gel, electrophoretically separated in 1xTAE buffer for 20 min at 120 V and detected under ultraviolet light (UV).

The alleles were identified by pyrosequencing on a PSQ96MA instrument using PyroMarkGold Q96 Reagents kit (50 x 96) (Qiagen, USA) on-line.

### Slaughter traits

Against the background of the evaluation of *H-FABP* as a candidate gene for intramuscular fat content, a control slaughter of young stock at 4 months of age (n=50) was carried out (in accordance with the requirements of the GOST 31777-2012). All animals studied 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. The character of the adipose tissue distribution in the slaughter material of youngsters was evaluated.

## Statistical analysis

Digital research materials were subjected to biometric processing. 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; ns = not significant at  $P < 0.95$ ). Student's  $t$ -test was applied for the statistical analysis (Johnson and Bhattacharyya 2010). The mean of a set of measurements was calculated

according to the formula:  $\bar{x} = \frac{\sum_{i=1}^n x_i}{n}$ , where  $\bar{x}$  is a mean

value;  $\sum_{i=1}^n x_i$  is the sum of all  $x_i$  with  $i$  ranging from 1 to  $n$ ,  $n$  is the number of measurements. The residual variation is expressed as a root mean square error (*r.m.s.e.*):

$\sigma = \sqrt{\frac{\sum_{i=1}^n (x_i - \bar{x})^2}{n-1}}$ . The standard error of mean (*s.e.m.*) was

calculated using the formula:  $s.e.m.(\bar{x}) = \frac{\sigma}{\sqrt{n}}$ . The reliability of

a sample difference (Student's  $t$ -distribution) was estimated by the test of the difference validity, which is the ratio between the sample difference and the non-sampling error. The test of the difference validity was determined by the

formula:  $t = \frac{x_1 - x_2}{\sqrt{s.e.m._1^2 + s.e.m._2^2}} \geq t_{st} (d.f. = n_1 + n_2 - 2)$

where  $t$  is a Student's  $t$ -distribution;  $x_1 - x_2$  is the difference of the sample mean measurements;  $\sqrt{s.e.m._1^2 + s.e.m._2^2}$  is the sample difference error;  $s.e.m._1$  and  $s.e.m._2$  are the nonsampling errors of the compared sample statistics;  $t_{st}$  is the standard criterion according to the  $t$ -table for the probability threshold preset depending on degrees of freedom;  $n_1$  and  $n_2$  are the numbers of measurements in the samples compared;  $d.f.$  is the degrees of freedom for the difference of two mean measurements.

## Results

Relatively young Kalmyk fat-tailed sheep breed was bred by reproductive crossbreeding between cross-bred fat-tailed ewes (Kalmyk-Edilbay) of different generations of Astrakhan and Kalmyk populations and rams of the Torgut breed imported from China and then rigorously selected in terms of the strain desired, which resulted in coarse wool animals that were rather large, of a strong constitution, proportional physique with developed bones and well-developed meat forms. The head was of medium size and arched faced; the neck was of medium length, the withers were wide and the chest is deep and wide. Fat elevated was of medium size. Fleece was of plait structure. Wool was of

medium thickness and fineness with half-luster shine. Crimps were large, slightly undulating. The fleece's main colour was white, additional one was on the ears and muzzle. Fertility and milk yield of the ewes was medium.

The Edilbay sheep breed is about two centuries old. It is characterized by outstanding meat productivity and early maturity. The breed was bred in the 19<sup>th</sup> century in the Russian Empire (on the territory of present-day Kazakhstan) by crossing Kazakh fat-tailed sheep and coarse wool sheep from the Astrakhan province. The local population needed a breed that could endure both drought cold winters and frosts. It also had to be capable to cover long distances and feed on sparse grass. These methods enabled selecting the most viable and large individuals, which resulted in the Edilbay breed being extremely unpretentious in keeping, high endurance and meat production, but not very prolific and with coarse wool. In 1992, animals of this breed were re-imported from Kazakhstan to the Volgograd region of the Russia Federation and widespread in the republics of the North Caucasus, Kalmykia, Astrakhan, Rostov and other regions. There are 16 breeding plants working with the breed, 4 of them are in the Volgograd and Astrakhan regions and other are in 8 subjects of the Russian Federation.

Both breeds have high meat and fat characteristics. However, their balance and qualitative improvement with respect to the market conditions and economy can be optimized by selection among other measures.

The quality of meat products is largely determined by the content of adipose tissue, its characteristics and localization. In our investigation, in order to assess the fat distribution in the body of the experimental lamb of the fat-tailed Kalmyk and Edilbay breeds, a control slaughter of young animals was carried out, with the weight of subcutaneous, fat tail, intramuscular and internal fat contained in the body of young animals being studied for quantitative evaluation (Table 2).

The rump was found to be the main place of adipose tissue accumulation in the body structure of the youngsters in experimental groups. The fat proportion in this structure of the body was 67.1% for Edilbay lamb and 64.8% for their counterparts of the Kalmyk fat-tailed breed. In absolute terms, the difference between the groups was 450 g. The superiority of the Edilbay lamb was also noted in terms of the weight of fat in the carcass. In absolute terms, it was 250 g or about 33%.

The youngsters of Kalmyk fat-tailed breed were superior in terms of the weight of intramuscular and internal fat. So, the weight of their intramuscular fat was by 140 g more and of internal one by 40 g, or by 27.5 and 33.3%, respectively.

Ultimately, the young sheep of Edilbay breed exceeded their peers of the Kalmyk fat-tailed breed with respect to the total weight of adipose tissue by more than 0.5 kg or 10%. If we compare the weight of fat and pre-slaughter live weight, we should note that the proportion of

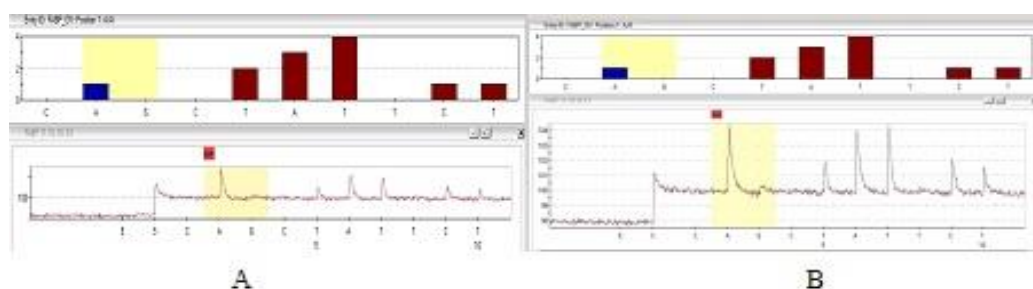
**Table 1:** Sequence of oligonucleotide primers

<i>FABP4</i> gene	Primer	Fragment length, n.p.
FAPli point	F: 5'-AAACTGGGATGGGAAATCAACC-3', R: 5'-TGCTCTCTCGTAAACTCTGGTAGC-3'	109

**Table 2:** Distribution of adipose tissue in the slaughter material of young sheep

Parameter	Unit	Kalmyk	Edilbay	
Pre-slaughter live weight	kg	41.7 ± 0.52**	38.0 ± 0.86	
Carcass weight	kg	18.3 ± 0.55*	16.2 ± 0.32	
Fat content in carcass	Subcutaneous	kg	0.75 ± 0.06	1.00 ± 0.06
	%		4.09	6.12
Fat tail	kg	2.87 ± 0.04	3.32 ± 0.03	
	%	15.58	20.49	
Intermuscular	kg	0.65 ± 0.07	0.51 ± 0.06	
	%	3.55	3.15	
Total	kg	4.27 ± 0.05	4.83 ± 0.09	
	%	23.33	29.81	
Suet	kg	0.16 ± 0.02	0.12 ± 0.02	
TOTAL (% of carcass)	kg	4.43 ± 0.04	4.95 ± 0.07	
	%	24.20	30.56	

Note: mean ± s.e.m.; comparisons between the two breeds based on a multivariate analysis of variance (ANOVA); \* $P < 0.05$ , \*\* $P < 0.01$

**Fig. 1:** Detection of the *FABP4* gene in: (A) Edilbay breed; (B) Kalmyk fat-tailed breed

fat in Kalmyk fat-tailed lamb was 4.43/41.7 or 10.6% and in Edilbay lamb, it was 4.95/38.0 or 13%.

From the economic point of view, the additional energy costs of feed for the fat production considerably increase the cost of mutton production. Negative characteristics are also provided by nutritionists, since an increase in fat in the carcass leads to an increase in the caloric content of such a product; recently the problems of obesity have been increasingly worrying the humanity. However, one should not interpret the established relationships unambiguously. The peculiarities of the national cuisine of many ethnic groups of people and their taste preferences against the background of a low level of lamb production in the world as a whole create good prospects for the demand for various quality groups of this product.

The taste, nutritional preferences and economic parameters should be taken into account when developing breeding programs for work with different breeds. The gene marker selection is a good help in this case. With this in mind, the main goal of our research was to study the *FABP4* gene polymorphism in coarse-haired sheep.

In our study, only the homozygous AA genotype was detected in the sheep population of the Edilbay and Kalmyk

breeds; the AG and GG genotypes were not identified. The *FABP4* locus appeared to be monomorphic in these populations (Fig. 1A and B).

## Discussion

There are works devoted to the study of the gene polymorphism of the fatty acid binding protein (*H-FABP*) and its influence on the economic traits of pigs. So, the polymorphism was detected for types D and H of the *H-FABP* gene in the Large white, Urzhum and White short-eared pig breeds. The proportions of the preferred DD and HH genotypes varied depending on the breed and made from 71.9 to 100% for Large white pig breed, 92.4% for Urzhum one and 89.8% for White short-eared breed (Arsienko and Gladyr 2002).

The studies of Chinese indigenous sheep breed (Xu *et al.* 2011) found polymorphic *FABP4* gene variants being associated with the meat tenderness. Along with qualitative parameters, quantitative values were considered; however, the correlation between the genotypes desired and main indices of meat production, *i.e.*, as pre-slaughter weight, slaughter yield of carcass and weight of the fresh carcass was not detected. Therefore, the *FABP* gene was

recommended to be regarded as a molecular marker associated with the tenderness of meat.

The research on the sheep of Edilbay and Kalmyk fat-tailed breeds with respect to the *FABP4* gene established that only its homozygous form was present. Perhaps this was due to the peculiarity of the genetic structure of this sample. Studies previously conducted on Barki sheep breed (Ibrahim et al. 2014) showed a considerable effect on tenderness and meat yield. This fact confirmed the hypothesis that the effect of the *FABP4* gene depends on the genetic potential of a particular breed and peculiarities of the physiological processes.

## Conclusion

The heterogeneity of Kalmyk fat-tailed sheep and, especially, Edilbay breed can be expected in Russia due to the diversity of breed resources used to create the Russian population of the Edilbay breed, as well as develop new approaches in breeding one of the most popular Russian sheep breeds widespread throughout the country.

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