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Original Article

Effect of DGAT1 gene polymorphisms in coarse-haired fat-tailed lambs of different genotypes

Efeito dos polimorfismos do gene DGAT1 em cordeiros de pelo grosso e cauda gorda de diferentes genótipos

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Abstract

The aim of this study was to investigate the DGAT1 gene polymorphism and its effects on lamb weight in kazakh and tajik sheep breeds. A total of 97 blood samples were collected from purebred (edilbay × edilbay) and crossbred lambs (edilbay × gissar) breed by the Baiserke Agro Scientific and Production Center in the Talgar District of the Almaty Region of Kazakhstan. Animals were genotyped for DGAT1-*Alu*I polymorphism using the polymerase chain reaction-restriction length polymorphism (PCR-RFLP) method. The result of PCR-RFLP showed that purebred (edilbay × edilbay) sheep had three genotypes (CC, CT and TT) and crossbred sheep had two genotypes (CC and CT). The predominant genotype was CC with a frequency of 0.70 and 0.58 in purebred sheep and crossbred sheep breeds, respectively. The DGAT1 gene showed no significant association with live weight of lambs at different times in both breeds studied. However, the study showed that the CC genotype produced higher live weight at day 60 in purebred sheep (CC: 33,668 kg and CT: 32,444) and at day 120 (CC: 41,487 and CT: 40,929) in crossbred lambs. The present study was the first to investigate the polymorphism and relationships between genotypes and lamb live weights for DGAT1 gene in sheep breeds, purebred and crossbred. We conclude that further comprehensive investigations should be done for the exact evidence of the effects of DGAT1/Aluı polymorphism on lamb live weights.

Keywords: Edilbay, gissar, purebred, crossbred, lamb weight, DGAT1 gene, AluI, PCR-RFLP.

Resumo

O objetivo deste estudo foi investigar o polimorfismo do gene DGAT1 e seus efeitos no peso do cordeiro em duas raças de ovelhas cazaques e tadjiques. Um total de 97 amostras de sangue foram coletadas de cordeiros de raça pura (edilbay × edilbay) e mestiços (edilbay × gissar) criados pelo Baiserke Agro Scientific and Production Center no distrito de Talgar da região de Almaty do Cazaquistão. Os animais foram genotipados para o polimorfismo DGAT1-AluI usando o método de reação em cadeia da polimerase-polimorfismo de comprimento de restrição (PCR-RFLP). O resultado da PCR-RFLP mostrou que ovelhas de raça pura (edilbay × edilbay) tinham três genótipos (CC, CT e TT) e ovelhas mestiças tinham dois genótipos (CC e CT). O genótipo predominante foi CC com uma frequência de 0,70 e 0,58 em ovelhas de raça pura e mestiças, respectivamente. O gene DGAT1 não mostrou associação significativa com o peso vivo dos cordeiros, em diferentes momentos, em ambas as raças estudadas. No entanto, o estudo mostrou que o genótipo CC produziu maior peso vivo no dia 60 em ovelhas de raça pura (CC: 33,668 kg e CT: 32,444 kg) e, no dia 120, (CC: 41,487 kg e CT: 40,929 kg) em cordeiros mestiços. O presente estudo foi o primeiro a investigar o polimorfismo e as relações entre genótipos e pesos vivos de cordeiros para o gene DGAT1 em raças de ovelhas, puras e mestiças. Concluímos que investigações mais abrangentes devem ser feitas para a evidência exata dos efeitos do polimorfismo DGAT1/AluI nos pesos vivos dos cordeiros.

Palavras-chave: Edilbay, gissar, raça pura, raça mestiça, peso de ovelha, gene DGAT1, AluI, PCR-RFLP.

1. Introduction

The edilbay sheep was created at the end of the 19th century in the West Kazakhstan region through folk selection. The breed occupies one of the first places among all breeds in the world in terms of live weight and level of meat and fat productivity, as well as the unsurpassed precocity of young animals (Karabassova et al., 2022). The number of edilbay sheep in Bayserke Agro LLP in Almaty region is about 7,000 heads. The edilbay is the main improving breed of fat-tailed sheep in the Republic of Kazakhstan, the proportion of which is currently about 80%. The average live weight of ewes of the edilbay breed under farm conditions was 68 kg, rams 110-115 kg; rams

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at the age of 4-4.5 months, that is, after beating them from the queens - 39.6 kg, lambs 36.3 kg, and the live weight of two rams of the gissar breed brought to the farm is 120-130 kg.

The gissar breed of sheep is a highly specialized meat and fat breed of sheep, which was created through folk selection over many centuries and is currently bred in Tajikistan and Uzbekistan in dry and hot climates (Ali et al., 2024; Zaitsev et al., 2024). The gissar breed of sheep has the highest live weight and meat-fat qualities in the world (Abrantes et al., 2023). A distinctive feature of the breed is its high hereditary biological and economic precocity (Seitkamzina et al., 2023), which is not inferior to the best breeds of sheep in the world (Buienbayeva et al., 2023). The edilbay breed of sheep has a strong constitution, good adaptability to year-round grazing, unsurpassed early maturity and high live weight. Gissars are a highly specialized meat-fat fat-tailed breed of sheep created by folk selection on the territory of Tajikistan. It is characterized by a slightly higher live weight compared to sheep of the edilbay breed, a higher meat yield and high-quality tail fat.

The main objective method characterizing the growth of an animal is the change in live weight at different age periods. Live weight is an important trait for animals because it is positively correlated with many economically useful traits (Afolayan et al., 2006; Karynbayev et al., 2023; Akhylbekova et al., 2022). Animal weight, like all quantitative traits, is determined not only by the type of feeding (Baiseitova et al., 2024; Lapshin et al., 2023) and rearing system, but also by the genotype of each specific animal (Santos-Silva et al., 2002; Ombayev et al., 2024). Identification of livestock genetic structure using quantitative trait locus (QTLs) associated with economic traits such as milk and meat has been investigated in mapping studies (Khan et al., 2021).

The diacylglycerol O-acyltransferase 1 gene (DGAT1) is one of the most studied candidate genes for dairy and meat production traits. Several polymorphisms associated with meat and milk production traits and live weight have been reported in the DGAT1 gene in sheep (Amri et al., 2024; Bayraktar and Shoshin, 2022; Dai et al., 2022; Dervishi et al., 2015). However, very limited studies have been conducted in fat-tailed sheep breeds on the association of the DGAT1 gene with live weight in purebred sheep and crossbred lambs. Therefore, the current study aimed to investigate the AluI polymorphism on exon 17 of the DGAT1 gene and its effects on live weight of lambs at 30, 60 and 120 days of age.

2. Materials and Methods

2.1. Animals, phenotypes and DNA isolation

In this study, 97 coarse-wool fat-tailed lambs were used as the research material, of which 67 lambs (Edilbay × Gissar) were crossbreds, 30 lambs were purebreds (Edilbay × Edilbay). The study was conducted at the educational and scientific production center "Baiserke-Agro" in the Talgar district of the Almaty region of Kazakhstan. The phenotypes were registered in the same farm. Lambs were born from ewes who have reached 3.5 years of age. Lambs were weighed at birth and on days 30, 60 and 120 and blood samples were taken on day 120 from the jugular vein into tubes under veterinary supervision in accordance with local regulations. Blood samples were frozen at -20 °C immediately after sampling for optimal preservation of the samples and delivered to the laboratory in a frozen form. Isolation of DNA from blood was carried out in the "Kazakh-Japanese" center of the Kazakh National Agrarian Research University. DNA was isolated from whole blood according to the method provided by the the DNA-Sorb-B reagent kit.

2.2. Amplification of the DGAT1 gene and genotyping

To amplify a fragment of 309 bp of of DGAT1 was used the primers; Forward: 5′-GCATGTTCCGCCCTCTGG-3′ and Reverse: 5′- GGA GTC CAA CAC CCC TGA-3′. PCR conditions for the DGAT1 gene consisted of pre-denaturation (at 95 °C for 5 min) and 35 cycles consisting of denaturation at 95 °C for 5 min, at 60 °C for 30 s, at 72 °C for 30 s. Finally, postelongation was carried out at 72 °C for 10 min. The reaction mixture for PCR of the DGAT1 gene was prepared in a final volume of 25 µl, and the PCR products (309 bp) were digested using 1-2 U restriction enzyme *Alu*I with incubation for 1 h at 37 °C. Digestion fragments were run on agarose gel electrophoresis (%2) and then checked for genotypes under UV transylmunator. To determine the genotypic structure of the studied animals, all PCR products were subjected to a cleavage process using 5 units of *Alu*I restriction enzyme.

2.3. Statistical analysis

General Linear Model (GLM) was used to investigate the association between live weights at different ages and identified genotypes in the study. The analysis was performed in Minitab program. The used model was (Equation 1):

$$Yijk = \mu + \alpha i + \beta j + \alpha\beta ij + eij \quad (1)$$

where: *Yijk*: traits measured; μ: overall mean for each trait; αi: gender effect; βj: genotype effect (CC and TC); αβij: interaction between genotype and gender; *eij*: random error.

The frequency values of identified allele and genotype and the Chi square test X^2 were calculated by popgene program (Yeh et al., 2000).

3. Results

3.1. DGAT1 gene polymorphism

A fragment of 309 bp was successfully amplified for the DGAT1 gene and all PCR products were subjected to the digestion of *Alu*I enzyme. After PCR-RFLP analysis, two alleles (C and T) and three genotypes (CC, CT and TT) were obtained in the study as given in Figure 1.

CC genotype was a fragment of 309 bp; CT genotype was two fragments of 309 and 272 bp; TT genotype was only 272 bp fragment. In the study, TT genotype was only observed in purebred lambs, but not in crossbred lambs. The DGAT1 gene was found to be polymorphic

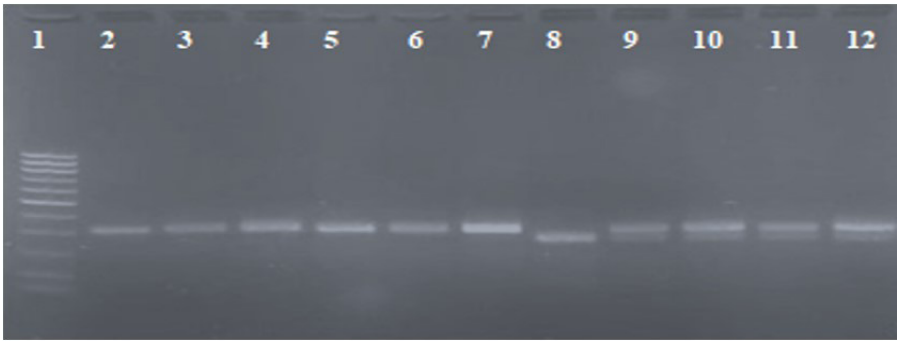

Figure 1. PCR-RFLP analysis of DGAT1 gene locus. L1: 50 bp ladder; L2-7: CC genotypes; L8: TT genotype; L9-12: CT genotype.

Table 1. Allele and genotype frequencies of DGAT1 gene.

Breed	N	Allele frequency		Genotype frequency			Heterozygosity			P-value
		C	T	CC	CT	TT	Ho	He	X^2	
Purebred lambs (edilbay × edilbay)	30	0.83	0.17	0.70	0.27	0.03	0.2667	0.2825	0.103111	0.748128
Crossbred lambs (edilbay × gissar)	67	0.79	0.21	0.58	0.42	0.00	0.4179	0.3331	4.483019	0.033423

N: number of individuals; Ho: observed heterozygosity; He: expected heterozygosity; X^2: chi-square test.

Table 2. Association analysis between DGAT1 gene genotypes and live weights.

Breed	Genotype	Age Weights			
		Birth (X ± SEM)	30th (X ± SEM)	60th (X ± SEM)	120th (X ± SEM)
Purebred lambs (edilbay × edilbay)	**CC**	5,876 ± 0,116	8,381 ± 0,090	33,668 ± 0.698	39,000 ± 0.644
	CT	5,844 ± 0,129	8,300 ± 0,090	32,444 ± 0.766	39,111 ± 0.696
	P	0,640	0,793	0.758	0.788
Crossbred lambs (edilbay × gissar)	**CC**	5,782 ± 0,094	8,269 ± 0,082	33,718 ± 0.469	41,487 ± 0.483
	CT	5,821 ± 0,110	8,439 ± 0,099	33,786 ± 0.548	40,929 ± 0.471
	P	0,670	0,054	0.221	0.649

in both crossbred and purebred lambs. According to the results of the Chi-square (X^2), purebred lambs sheep was in Hardy – Weinberg equilibrium (P>0.05) while not for crossbred lambs (P<0.05) (Table 1). Allele and genotype frequency was 0.83 (C) and 0.17 (T); 0.70 (CC), 0.27 (CT) and 0.03 (TT) for purebred lambs while was 0.79 (C) and 0.21 (T); 0.58 (CC) and 0.42 (CT) for crossbred lambs.

It was found that CC genotype' frequency was the highest for both studied breeds. Heterozygous genotypes were higher in crossbred lambs, whereas TT gonotype was not observed in the investigated crossbred lambs.

3.2. Association analysis

The results of the association analysis of the DGAT1 gene genotypes with the live weight of lambs at different ages are summarized in Table 2. Purebred and crossbred lambs with CC genotype had a higher live weight and average than lambs with other genotypes at different ages (60th and 120th, respectively).

4. Discussion

4.1. Polymorphisms of ovine DGAT1 gene

The allele and genotypic frequency results are shown in Table 1, as are the Hardy Weinberg results and heterozygosity values. The crossbred sheep was not in Hardy – Weinberg equilibrium by X^2 value (P < 0.03) due to some factors that affects allelic distrubuition such as selection and crossbreeding (Ala Noshahr and Rafat, 2014). This is agreementconsistent with the results of various studies (Mohammadi et al., 2013; Xu et al., 2009). The purebred lambs was in Hardy - Weinberg equilibrium. Similar results were also observed in the studies of Bayraktar and Shoshin (2022), who examined Awassi sheep, and Dervishi et al. (2015) examined a total of 9 sheep breeds (Bayraktar and Shoshin, 2022; Dervishi et al., 2015).

In the study, tree genotypes of DGAT1 gene was observed in purebred lambs by PCR-RFLP method, while TT genotype was no observed in crossbred lambs. Among

the studied purebred lambs, only one individual carried the TT genotype. It was reported that Barki, Najdi and Harri sheep breeds had no TT genotypes. In the present study, the most common allel was C with the frequency of 0.83 (Edilbay) and 0.79 (Gissar). Similarly, the highest frequency with 0.88 was reported for Barki breed. In contrast to this result, T allel was the highest frequency in the breeds of Moghani, Zel and Lori-Bakhtiari (Ala Noshahr and Rafat, 2014; Mohammadi et al., 2013).

It is important point out that the currenty study was the first to investigate the genetic structure of purebred and crossbred lambs in terms of DGAT1 gene. Therefore, it was not possible to compare the findings obtained with other Kazakh breeds. The edilbay breed, which has been selected by local Kazakh inhabitants since 1930s, is characterized by outstanding meat production. A study of CAST gene at the sheep of edilbay breed demonstrated that sheep with AB genotype was set as desirable in terms of meat quality (Kolosov et al., 2021).

4.2. Association analysis between DGAT1 gen polymorphism and live weights

The DGAT1 gene is an important candidate gene that is frequently studied in livestock animals for growth and meat quality traits as well as milk production and traits (Bayraktar and Shoshin, 2022; Dai et al., 2022; Khan et al., 2021; Sadeghi et al., 2020; Tăbăran et al., 2014). However, studies examining the effects of the DGAT1 gene on live weight of Kazakh sheep breeds are quite rare. In the present study, lambs with CC genotype showed higher live weight compared to other genotypes for different ages. Although no significant differences between genotypes in live weight were observed in purebred lambs, animals with CC genotype gained 1.2 kg more live weight at 60 days of age compared to other genotypes. Similarly, crossbred animals with CC genotype showed a live weight gain of about half a kilogram (0.558) compared to other genotypes. Lambs with CC genotypes from the Akkaraman sheep breed in Türkiye were reported to have higher live weight than lambs with other genotypes, especially at day 60. This finding was consistent with our study results. Another study using Lori-Bakhtiari and Zel sheep breeds from Iran found that animals with the CC genotype had higher carcass yields than those with another genotype (Mohammadi et al., 2013). In contrast to these results, Indonesian sheep showed that the CT genotype had the highest value of carcass traits and about 2 kg more hot carcass weight compared to the CC genotype (Amri et al., 2024). Likewise, Barki lambs showed higher live weight compared to other genotypes at birth and weaning age (Abousoliman et al., 2020). The results obtained from the present study suggest that application of selection using genomic information could be effective in the studied herd. However, the relatively small sample size represents a certain limitation for marker-assisted selection. Nevertheless, the results obtained showed that both breeds examined have a variation in the DGAT1 gene, which is important for selection studies.

5. Conclusion

This study was the first to investigate the genetic structure of the DGAT1 gene and its effects on live weight in purebred lambs and crossbred lambs from Kazakhstan. The results showed that both breeds had genetic variation in the DGAT1 gene, allowing further association studies. In the present study the DGAT1 gene had no significant influence on the live weight of the lambs in various respects. However, CC-genotyped animals showed a tendency towards an increase in live weight at different ages in both breeds examined. To obtain more reliable results, further studies should be carried out on the breeds of edilbay and gissar lambs.

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