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Research Article

DGAT1, CAST and IGF-I Gene Polymorphisms in Akkaraman Lambs and Their Effects on Live Weights up to Weaning Age

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Abstract

Early live weight in sheep is important for lamb survival and average weight gain until slaughter. The aim of the present study was to investigate association between *CAST-Msp*I, *DGAT1-Alu*I and *IGF-1-Bsp*143II polymorphisms and early live weights between birth and weaning age in Akkaraman lambs. A total of 374 lambs were genotyped for *CAST-Msp*I, *DGAT1-Alu*I and *IGF-1-Bsp*143II polymorphisms by the polymerase chain reaction and restriction length polymorphism (PCR-RFLP) method. The results of PCR-RFLP analysis showed that the SNPs had three genotypes of *CAST-Msp*I polymorphism, two genotypes of *DGAT1-Alu*I polymorphism and one genotype of *IGF-1-Bsp*143II polymorphism of these, *CAST-Msp*I-MM, *DGAT1-Alu*I-CC and *IGF-1-Bsp*143II-BB were the predominant genotypes in the Akkaraman sheep breed. The result of Chi-square analysis indicated that the Akkaraman sheep breed was in Hardy-Weinberg equilibrium for the investigated polymorphic genes. At the *DGAT1* locus, the CT genotype showed significantly heavier birth weight (P=0.044) compared to CC genotype. *CAST* gene did not show any association for the investigated traits. The results of this study demonstrate that the CT genotype had a positive effect on birth weight in Akkaraman sheep. We concluded that further investigations are needed in *DGAT1-Alu*I polymorphism and live weight at different ages in sheep.

Keywords: *Birth weight, DGAT1, Polymorphism, RFLP, Sheep*

Akkaraman Kuzularında DGAT1, CAST ve IGF-I Gen Polimorfizmleri ve Sütten Kesim Yaşına Kadarki Canlı Ağırlık Artışı Üzerine Etkileri

Öz

Bu çalışmada, Akkaraman ırkı kuzularda, *CAST-Msp*I, *DGAT1-Alu*I ve *IGF-1-Bsp*143II polimorfizmleri ile bu polimorfizmler ve bazı canlı ağırlıklar arasındaki ilişkilerin araştırılması amaçlanmıştır. PCR-RFLP yöntemi ile toplam 374 kuzu *CAST-Msp*I, *DGAT1-Alu*I ve IGF-*1-Bsp*143II polimorfizmleri yönünden genotiplendirilmiştir. Akkaraman ırkı koyunların, *CAST-Msp*I ve *DGAT1-Alu*I polimorfizmleri yönünden polimorfik iken *IGF-1-Bsp*143II polimorfizmi yönünden monomorfik oldukları görülmüştür. Akkaraman ırkı koyunlarda *CAST-Msp*I-MM, *DGAT1-Alu*I-CC ve *IGF-1-Bsp*143II-BB genotiplerinin en yaygın genotipler olduğu görülmüştür. Ki-kare test sonuçlarına göre Akkaraman ırkı koyunların *CAST-Msp*I ve *DGAT1-Alu*I polimofizmleri yönünden Hardy-Weinberg (HW) dengesinde oldukları görülmüştür. *DGAT1* lokusu yönünden TC genotipli kuzular, diğer genotiplilerle karşılaştırıldığında bu genotipteki kuzuların daha yüksek doğum ağırlığına (P<0.05) sahip oldukları görülmüştür. Bu çalışma sonunda Akkaraman koyun ırkında TC genotipinin doğum ağırlığı üzerine pozitif etkisinin olduğu görülmüştür. Çalışma sonunda *DGAT1-Alu*I polimorfizminin farklı yaşlardaki kuzularda canlı ağırlıkla arasındaki ilişkilerin araştırıldığı çalışmaların planlanmasına ihtiyaç olduğu düşünülmüştür.

Anahtar sözcükler: *Doğum ağırlığı, DGAT1, Polimorfizm, RFLP, Koyun*

INTRODUCTION

The United Nations Food and Agriculture Organization (FAO) estimates that food demand in the coming 50 years will double globally [1]. Also, the global meat demand in 2030 is projected to be 68% more than that of 2000 [2]. Therefore, it is suggested that it will be advantageous to determine specific genes in order to choose animals

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bearing the desirable alleles to improve existing breeds or populations [3].

According to FAO data, Turkey ranks 7th globally in terms of total sheep population with a stock of approximately 31 million [4]. Of the current sheep population of Turkey, 45% consists of a fat-tailed breed called the Akkaraman, which is bred in the central Anatolia [5]. The Akkaraman breed meets most of the mutton demand in Turkey in spite of low-quality pasture and poor climatic conditions, to which the breed has adapted well [6]. Studies report lamb birth weights ranging from 3.81 kg [6] to 4.56 kg [7] in Akkaraman sheep breed. They also-demonstrate that the Akkaraman breed manifests considerable variations in terms of birth weight. Therefore, studies aimed at improving the lamb birth weights and growth characteristics will be important for this breed.

Quantitative genetic studies done on sheep demonstrated that some genetic factors have an effect on lamb birth weight. The heritability of lamb birth weight in diverse sheep breeds is reported to be between 0.15 and 0.24 [8]. Therefore, the application of genomic selection is considered as a potentially successful approach for the improvement of lamb birth weights [9]. Despite the minor effect on livestock breeding, some candidate genes to be used in the improvement of some polygenic characteristics such as growth have been reported to assist in the accurate estimation of the genetic value of different livestock species including sheep [10,11].

Diacylglycerol acyltransferase1 *(DGAT1),* is an enzyme, which takes part in the synthesis of triglycerides in adipocytes [12]. The *DGAT1* gene, encoding this enzyme is expressed in many tissues but predominantly in the adipose tissue and in the small intestine [13]. Studies have reported an association between the *DGAT1* gene and fat accumulation in sheep and cattle carcasses [14,15]. *DGAT1* gene has been found as a putative candidate gene for the milk fat content in sheep [16]. However, studies investigating the association between the SNPs in the *DGAT1* gene and mutton productivity are scarce. In one of these studies with the native Moghanian Iranian sheep breed, an association between the polymorphism in the 17th exon of the *DGAT1* gene and carcass weight was reported previously [17].

For livestock breeding, the calpastatin *(CAST)* gene has been stated to warrant attention in studies on improving live weight gain and meat quality [18]. The calpain-calpastatin system, consisting of three members, namely μ-calpain, m-calpain and calpastatin, takes part in many crucial processes in various tissues including muscle development [19,20]. Therefore, the *CAST* gene is considered to be a notable candidate gene for muscle growth and meat quality improvement in livestock breeding [21]. The *CAST* gene, located on the autosomal 5th chromosome of sheep, was reported to be associated with growth and live weight gain in a variety of sheep breeds [22].

The *IGF-I* protein, a member of the superfamily of insulin-like growth factor (IGF), is an important protein involved in the fertility, embryogenesis, and growth of mammalians [23,24]. In cattle [23], pigs [25] and goats [26], an association between the *IGF-I* gene and live weight gain was reported. However, studies examining the relationship between the *IGF-I* gene and live weight gain are relatively limited. *IGF-I* gene was proposed as a candidate gene for growth and meat yield traits in the Makui sheep breed [24].

To the best of our knowledge, there is no study on the association between the *CAST*, *DGAT1* and *IGF-1* genes and early live weight in Akkaraman sheep breed which is commonly reared in Turkey. The aim of the present study was to investigate association between *CAST-Msp*I, *DGAT1-Alu*I and *IGF-1-Bsp143*II polymorphisms and early live weight traits in the Akkaraman sheep breed.

MATERIAL and METHODS

Animals, Phenotypes and Genotyping

A total of 374 Akkaraman male lambs were used in this study. Phenotypes were recorded in the same farm. Lambs were born from the ewes those were in age two. The lambs were weighted at birth, and on the 30th, 60th and 90th day (which was classed as the weaning day) and blood samples were collected at 90th day from *Vena jugularis* in K_3EDTA tubes. All experimental procedures were performed according to the guidelines of the Local Ethics Committee for Animal Experiments at Erciyes University (13.11.2013 and #13/130). For the PCR processes, genomic DNA was obtained from whole blood by the phenol-chloroform extraction method.

PCR mixtures for the *CAST*, *DGAT1* and *IGF-1* genes were prepared as 25 μL volumes using 1.5 mM $MgCl_2$, 200 μM dNTPs, 200 μM primers (primers sequences in *Table 1)*, 1×PCR buffer, 1 U Taq polymerase and 50 ng genomic DNA. The PCR conditions for the *CAST* gene consisted of pre-denaturation (at 95ºC for 5 min), followed by 35 cycles consisting of denaturation at 95ºC for 1 min, annealing at 62ºC for 1 min, 72ºC for 2 min and post-elongation at 72ºC for 8 min. The PCR products (622 bp) were digested using 5 U of appropriate restriction enzyme. The PCR conditions for the *DGAT1* gene consisted of pre-denaturation (at 95ºC for 5 min), followed by 35 cycles consisting of denaturation at 95ºC for 30 sec, at 60ºC for 30 sec, at 72ºC for 30 sec. Finally, a post-elongation at 72ºC for 10 min was performed. The PCR products (309 bp) were digested using 5 U of appropriate restriction enzyme. The PCR condition for the *IGF-1* gene consisted of pre-denaturation (at 94ºC for 6 min), followed by 30 cycles consisting of denaturation at 94ºC for 30 sec, annealing at 55ºC for 30 sec, at 72ºC for 30 sec. Finally post-elongation at 72ºC for 10 min was performed. The PCR products (294 bp) were digested using 5 U of appropriate restriction enzymes.

Table 1. *Primer sequences and restriction enzymes*

Gene	Primer Sequence	RE	Reference
CAST	F: 5′-TGG GGC CCA ATG ACG CCA TCG ATG-3′ R: 5′-GGT GGA GCA GCA CTT CTG ATC ACC-3′	*Msp*I	[19]
DGAT1	F: 5′-GCA TGT TCC GCC CTC TGG-3′ R: 5′-GGA GTC CAA CAC CCC TGA-3′	*Alu*I	[27]
IGF-1	F: 5′-TGA GGG GAG CCA ATT ACA AAG C-3′ R: 5′-CCG GGC ATG AAG ACA CAC ACA T-3′	*Bsp143*II	[28]

RE: Restriction enzymes

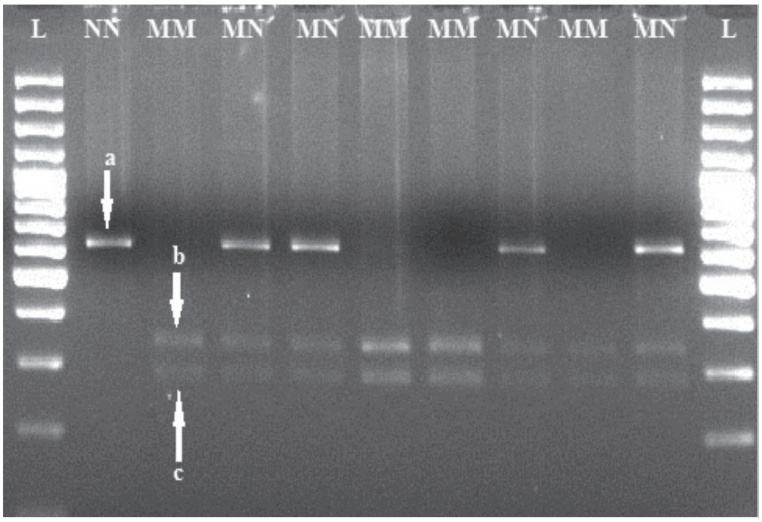

Fig 1. *CAST-Msp*I polymorphism pattern in 2% agarose gel electrophoresis (L; 100 bp DNA ladder; a: 622 bp band; b: 336 bp band; 286 bp band)

Statistical Analysis

Sheep phenotypic data were checked for normality before analyses with the UNIVARIATE procedure in SAS v9.0. (2004). Allele and genotype frequencies and Hardy-Weinberg equilibrium were calculated using the ALLELE procedure in SAS v9.0. (2004). Genotype-phenotype association was analysed with a generalized linear model using the GLM procedure in SAS v9.0 (2004). The effects of farm, feeding regime and age of ewes were not built into the linear model, because all animals were raised on the same farm therefore lambs did not expose to different environmental conditions. The reduced model included fixed effects of birth type (single or twin) and genotype. Genotypic comparisons were reported following Tukey-Kramer adjustment, and $P \leq 0.05$ was considered as significant.

The statistical model used as follows:

$$Y_{ij} = \mu + S_j + G_i + e_{ij}$$

Where Y_{ij} is the observation of the birth weight, 30th day, 60th day and 90th day live weight traits; μ is the overall mean for each trait, S_j is the fixed effect of j^{th} birth type, G_i is the fixed effect of i^{th} genotype for the relevant polymorphism and e_{ij} is the random residual error.

RESULTS

A 622 bp fragment for the *CAST* gene was amplified by PCR. After the digestion of PCR products with *Msp*I enzyme, three restriction patterns were obtained for the *CAST-Msp*I polymorphism. Two fragments (336 and 286 bp) were observed for the first pattern, which is called the MM genotype. For the second pattern, called the MN genotype, three fragments (622, 336 and 286 bp) were observed, while for the third pattern, only one fragment (622 bp) was observed for the NN genotype *(Fig. 1)*. The MM genotype frequency (0.81) was found to be the highest, while the NN genotype frequency (0.01) was the lowest. The M allele frequency (0.9) was higher than the N allele frequency *(Table 2)* and the examined Akkaraman population was found in Hardy-Weinberg equilibrium (HWE) for *CAST-Msp*I polymorphism *(Table 2)*.

A 309 bp fragment for the *DGAT1* gene was amplified, and PCR products were digested by *Alu*I enzyme. Two genotypes (CC and CT) were obtained for the *DGAT1-Alu*I polymorphism in the Akkaraman sheep breed in Turkey. Only one band (309 bp) was observed in the CC genotype. On the other hand, three bands (309, 272 and 37 bp) were expected in the CT genotype, but the 37 bp band could not be seen on 3% agarose gel electrophoresis because it was too small. However, two bands (309 and 272 bp) were found to be sufficient for genotyping of individuals *(Fig. 2)*. It was found that CC had the highest genotype frequency (0.91), whereas the TT genotype was not found in the investigated Akkaraman lambs. The population was found in HWE for the *DGAT1-Alu*I polymorphism *(Table 2)*.

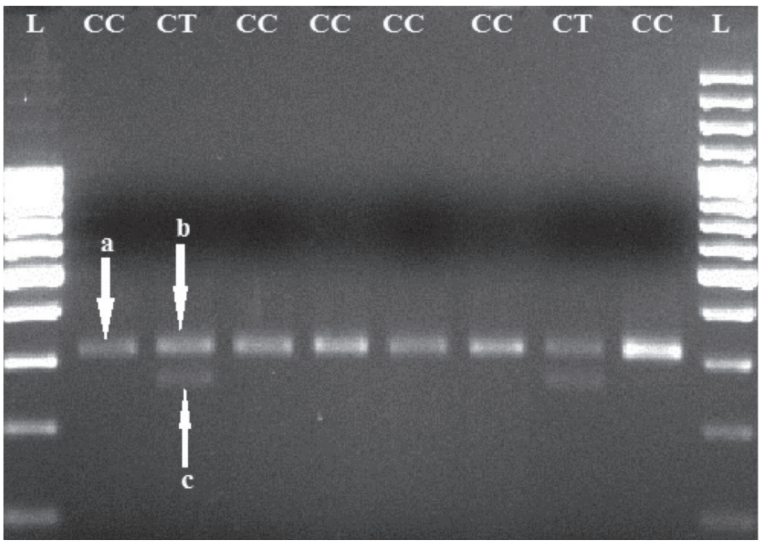

Fig 2. *DGAT1-Alu*I polymorphism pattern in 3% agarose gel electrophoresis (L; 100 bp DNA ladder; a: 309 bp band; b: 272 bp band)

Fig 3. *IGF-1-Bsp*143II polymorphism pattern in 3% agarose gel electrophoresis (L; 100 bp DNA ladder; a: 294 bp band)

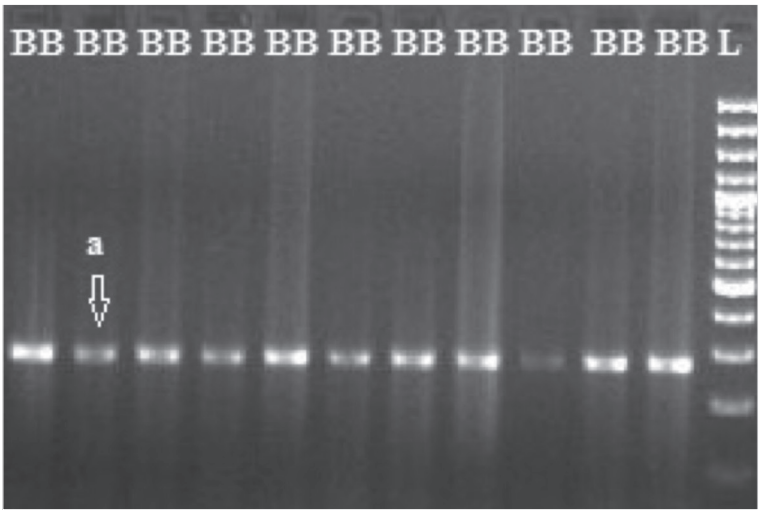

Table 2. *Allele and genotype frequencies of CAST, DGAT1 and IGF-1 in Akkaraman lambs*

Gene	n	Genotype						Allele Frequency		Chi-squared (df=1)
CAST	374	MM		MN		NN		M	N	X^2=0.93^{NS} P=0.3355
		Obs (Exp)	F	Obs (Exp)	F	Obs (Exp)	F	0.9	0.1	
		302 (303.66)	0.81	70 (66.68)	0.18	2 (3.66)	0.01			
DGAT	374	CC		CT		TT		C	T	X^2=0.75^{NS} P=0.3874
		Obs (Exp)	F	Obs (Exp)	F	Obs (Exp)	F	0.96	0.04	
		342 (342.68)	0.91	32 (30.63)	0.09	0 (0.68)	0			
IGF-1	374	AA		AB		BB		A	B	-
		Obs (Exp)	F	Obs (Exp)	F	Obs (Exp)	F	0	1	
		0	0	0	0	374	1			

Obs: *Observed genotype;* ***Exp:*** *Expected genotype;* ***F:*** *Frequency;* ***df:*** *degree of freedom;* ***X²:*** *Chi-square;* ***^{NS}:*** *Non-significant (P<0.01)*

A fragment of 294 bp was successfully amplified for the *IGF-1* gene and PCR products were digested with *Bsp*143II restriction enzyme. After digestion, only the B allele and BB genotype were found in the Akkaraman lambs *(Fig. 3)*.

Association analysis revealed that a significant difference was found among lambs with *DGAT1-Alu*I genotypes (CC and CT) in terms of birth weights (P=0.044). Lambs with *DGAT1-Alu*I-CT genotypes had higher birth weight compared to CC genotype individuals. On the other hand,

Table 3. *Means, standard errors of means (SEM), and statistical significance with CAST and DGAT1 genotypes for different age weight (kg) in Akkaraman lambs*

Gene	Genotype	Age Weight			
		Birth X±SEM	**30th day X±SEM**	**60th day X±SEM**	**90th day X±SEM**
CAST	MM	4.730±0.081	10.922±0.203	18.593±0.368	28.264±0.512
	MN	4.825±0.120	10.920±0.300	18.500±0.544	27.638±0.758
	NN	4.387±0.082	9.681±0.206	19.768±0.374	31.766±0.520
	P	**0.636**	**0.818**	**0.934**	**0.533**
DGAT1	CC	4.489±0.275	10.530±0.689	19.118±1.252	29.250±1.743
	TC	4.805±0.312	10.443±0.782	18.790±1.418	29.196±1.975
	P	**0.044**	**0.824**	**0.645**	**0.956**

no significant association was found between *CAST-Msp*I genotypes and early live weight traits in Akkaraman lambs *(Table 3)*.

DISCUSSION

Growth is a quantitative characteristic controlled by many genes. These genes can be linked to genetic markers and are segregated together [14]. An ideal marker is favourable if it is polymorphic, has a simple inheritance pattern and is easily identified [29]. This study investigated the relationship of *CAST-Msp*I, *DGAT1-Alu*I and *IGF-1-Bsp143*II polymorphisms, which are considered as potential markers for early live weight in sheep.

This study showed that genotype CC was the most common genotype (0.91) in terms of *DGAT1-Alu*I polymorphism and also revealed that the genotype TT was not present in Akkaraman lambs. The C allele frequency (0.96) was observed to be higher than that of T allele frequency, and the investigated samples were found to be in HW equilibrium for *DGAT1-Alu*I polymorphism. Similar to findings of this study, the genotype CC was reported to be the most common genotype in Imroz and Chios sheep breeds (0.68 and 0.52, respectively), which are bred in Turkey [30]. Additionally, while frequency of genotype CC was reported highest, genotype TT was found to be lowest in Indian [31], Iranian [32], Romanian [33], Chinese [27] and US [34] sheep breeds. Unlike our findings, there are a few studies reporting that the frequency of the TT genotype is higher than those of other genotypes [17].

The *DGAT1* gene has been extensively studied for the quality of meat in various livestock species. Studies examining the relationship of the *DGAT1* gene with characteristics of growth and live weight gain are relatively rare. One of these studies reported that individuals with *DGAT1-Alu*I-CC genotype had higher hot carcass weight and higher carcass yields than other genotypes in fat-tailed Lori-Bakhtiari sheep and in short-tailed Zel sheep bred in Iran [14]. It was reported that individuals with CC genotypes from the Moghani sheep breed in Iran were superior to those with other genotypes in terms of hot carcass weight and hot dressing percentage [17]. In the present study, it was found that lambs with CT genotypes had higher birth weights compared to other genotype in the examined animals (P=0.044). Birth weight is an important trait for the survival of lambs [35]. Lambs with birth weights of 4.36-4.77 kg were reported to have the maximum survival rates in the period from birth until weaning age [35].

In the current study genotype frequency of *CAST-Msp*I-MM genotype was found highest in Akkaraman sheep breed. *CAST-Msp*I-MM genotype frequencies was found also highest in other Turkish sheep breeds such as Akkaraman, Kıvırcık, Karacabey Merino, Imroz, İvesi and Çine Çaparı breeds, whereas, frequency of genotype MN was highest in the Sakız and Karya sheep breeds [36,37]. Similar to our findings *CAST-Msp*I-MM frequency was also found highest in different sheep breeds among the world [19,20,38-40].

Due to its role in the development of muscle cells, the *CAST* gene is considered to be an important candidate gene in monitoring the growth of livestock animals. The associations between *CAST* gene polymorphisms and live weights at different time points have been investigated. In lambs of the Romney breed raised in New Zealand, it was reported that there was a relationship the *CAST* gene and birth weight, but not with daily live weight gain [41]. A study reported the relationship between *CAST* genotypes and lamb birth weight and daily live weight gain [42]. In the Egyptian Barki sheep breed, an association between the *CAST* gene and fat free carcass yield was reported [43]. In the native Balkhi and Kajli sheep breeds in Pakistan, it was reported that individuals with the "MN" genotype were found to have more favourable daily live birth weights compared to those with other genotypes [22]. In the native Kıvırcık sheep breed in Turkey, individuals with the NN genotype were reported to have less favorable results in terms of daily live birth weight gains than those with different genotypes [36]. In the Russian breeds of the Soviet Merino and Salks, individuals with the MN genotype were reported to have more favorable results in terms of weaning weight and mean daily weight gain compared to

those with other genotypes [38]. However, there are studies reporting no association of the *CAST* gene with growth traits in diverse breeds of sheep [18,20]. In agreement with these findings, this study investigating the Turkish native Akkaraman breed demonstrated no relationship between *CAST-Msp*I polymorphism and birth weight or with weights at selected time points in the period after birth till weaning (day 90) in the examined samples.

The *IGF-I* gene has been reported to be a candidate gene for the growth traits of livestock animals [26]. Therefore, in this study, our aim was to investigate the relationship of the *IGF-1-Bsp*143II polymorphism with early live weight. However, unlike other genes, which were examined in this study, all of the 374 lambs of the Akkaraman breed in this study were found to have the monomorphic BB genotype in terms of the *IGF-1-Bsp*143II polymorphism. Therefore, the relationship of the *IGF-I* gene with weights at different time points could not be investigated. Unlike our study, it was reported that three different genotypes, namely AA, AB and BB, were determined in Han and Hu sheep breeds of China; AA and AB genotypes were present in the Texel breed and only AA genotype was present in the Dorset breed [28].

This is the first study investigating the *CAST-Msp*I, *DGAT1-Alu*I and *IGF-1-Bsp143*II polymorphisms altogether in Akkaraman sheep breed. It was determined that *CAST-Msp*I and *DGAT1-Alu*I polymorphisms are persistently present and are in HW equilibrium in the Akkaraman breed. It was also determined that the Akkaraman breed is monomorphic in terms of *Bsp143*II polymorphism. These results demonstrate that genetic variabilities persist in the Akkaraman breed. On the other hand, this study demonstrated the association between the *DGAT1-Alu*I polymorphism and birth weight in the Akkaraman sheep breed. However, it did not determine any association of the *CAST-Msp*I polymorphism with any of the investigated items in the study. Therefore, *DGAT1-Alu*I polymorphism is considered to be a potential molecular marker in the improvement of lamb birth weights in the Akkaraman sheep breed.

Conflicts of Interest

The authors declare no conflict of interest.

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