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Research Article

Associations Between MSTN/HaeIII Polymorphism and Reproductive and Growth Characteristics in Morkaraman Sheep

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Abstract: The myostatin gene inhibits skeletal muscle growth in advanced animals, and a mutation in the gene coding region increases muscle formation. Hence, it is accepted to be a candidate gene for the selection of some production traits. The objective of the current research was to examine the genotypes of the myostatin gene and reveal the associations between the genotypes and a number of traits, including birth weight, weaning weight, weaning age, average daily weight gain, and productivity, in 110 Morkaraman sheep. Genotypes were determined by the PCR-RFLP method using the *Hae*III restriction enzyme, and the genotype frequencies were found to be 9%, 69%, and 22% for MM, Mm, and mm in the myostatin locus, respectively. The *M* allele frequency was 44%, whereas the *m* allele frequency was 56%. In the studied population, the myostatin locus was not in Hardy-Weinberg equilibrium. The association analysis revealed no statistically significant impact of the *MSTN* gene polymorphism in exon 3 on birth weight, weaning weight, and productivity (P>0.05) but found a significant effect on weaning age and average daily weight gain (P<0.05). As a result, the *MSTN* gene showed polymorphisms in Morkaraman sheep and can be regarded as a genetic marker for sheep selection according to the association analysis results.

Keywords: *MSTN gene, Polymorphism, Morkaraman, PCR-RFLP, Production traits*

Morkaraman Koyunlarında MSTN/HaeIII Polimorfizmi İle Dölverimi ve Büyüme Özellikleri Arasındaki İlişkiler

Öz: Miyostatin geni, gelişmiş hayvanlarda iskelet kası büyümesinin bir inhibitörüdür ve gen kodlama bölgesindeki bir mutasyon kas oluşumunu arttırır. Bu nedenle bazı verim özelliklerinin ıslahı için aday gen olarak kabul edilmektedir. Çalışmada, Morkaraman koyunlarından alınan genomik DNA örneklerinden MSTN/*Hae*III gen polimorfizmine ait genotiplerin araştırılması, genotip ve alel frekanslarının dağılımının belirlenmesi ve genotipler ile doğum ağırlığı, sütten kesim ağırlığı, sütten kesim yaşı, ortalama günlük canlı ağırlık artışı ve dölverimi gibi bazı özellikler arasındaki ilişkilerin belirlenmesi amaçlanmıştır. Kayıtlı 110 Morkaraman koyunundan kan örnekleri alındı ve her bir örnekten DNA ekstrakte edildi. Genotipler, PCR-RFLP yöntemi ile myostatin geninin polimorfik ekson 3 bölgesi için *Hae*III restriksiyon enzimi kullanılarak belirlendi. Sonuç olarak, miyostatin lokusunda MM, Mm ve mm için genotip frekansları sırasıyla %9, %69 ve %22 idi. M allel frekansı %44 ve m allel frekansı %56 idi. Popülasyonda miyostatin lokusu Hardy-Weinberg genetik dengesinde değildi. Sonuçlara göre *MSTN* geni ekson 3 polimorfizminin etkisi, doğum ağırlığı, sütten kesim ağırlığı ve dölverimi üzerine istatistiksel olarak önemli bulunamadı (P>0.05), ancak, sütten kesim yaşı ve ortalama günlük canlı ağırlık artışı üzerine etkisi önemliydi (P<0.05). Sonuç olarak, *MSTN* geni Morkaraman koyunlarında polimorfizm göstermiştir ve bu polimorfizm ıslah çalışmalarında genetik markör olarak kullanılabilir.

Anahtar sözcükler: *MSTN geni, Polimorfizm, Morkaraman, PCR-RFLP, Verim özellikleri*

Introduction

In recent years, many researchers have been trying to reveal the genetic structure of breeds by using new techniques at the molecular level and conducting studies at the DNA level to protect animal breeds as a genetic resource and help the breeding of indigenous breeds [1-6]. Sheep breeding is performed worldwide, and meat yield represents an important part of the income from sheep breeding. It is essential to increase productivity and quality

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per animal but not the number of animals in animal breeding. Production traits, such as growth, milk yield, multiple births, and meat yield, represent the most crucial economic features in sheep breeding. Since many genes affect the mentioned production traits, their improvement takes a long time [7-11].

The production of sheep meat can be increased even further by researching the impacts of the myostatin gene on sheep meat production and making the said gene available in sheep breeding.

MSTN is the growth differentiation factor 8 (GDF8) gene and regulates skeletal muscle growth negatively [12]. The polymorphisms of the said gene have been studied in various goat and sheep breeds [12,13]. The size of the sheep *MSTN* gene is 6756 bp, and it is located on chromosome 2 [14]. The myostatin protein belongs to the tumor growth factor (TGF-β) superfamily, and its synthesis is performed by a 376-amino acid precursor protein containing three domains, such as a C-terminal domain, N-terminal propeptide domain, and a signal sequence [15]. There is an association between single nucleotide polymorphisms (SNPs) within the coding region of the myostatin gene and double muscle [15,16]. Since it is nutritionally and economically important, extensive research has been done on various methods with the objective of determining the carcass and production traits of domestic animals [3,4,11].

Many studies have indicated significant associations between the myostatin gene and some yield traits in sheep, e.g., a study on muscle development in Belgian Texel sheep [17] and New Zealand Texel sheep [16,18], studies on muscle and fat thickness in English Texel sheep [19], Charollais sheep [20], and Ujumqin sheep [21] and on increased carcass amount in Norwegian White sheep [3], increased body weight and weaning weight in Baluchi sheep [22], on growth and carcass features in New Zealand Romney sheep [4,23], and on lamb birth weight and skeletal muscles in Dorper and Hu ewes [24]. Molecular methods, e.g., PCR-RFLP and PCR-SSCP, research the genetic variants in the myostatin *(MSTN)* gene associated with meat traits [3,11]. *MSTN* gene polymorphisms are associated with meat production and decreased total lean meat and an increased proportion of loin meat [4]. Marker-assisted selection (MAS) assists with the correct selection of DNA variations related to differences in growth and carcass traits and, thus, the selection of individuals with superior characteristics. Hence, sequencing of the livestock myostatin gene is essential to understand the structure, function, and evolution of the gene and generate genomic resources for the advancement of knockout technology.

The Morkaraman breed, the native breed dominant in the eastern and northeastern regions of Turkey, represents a fat-tailed breed, which has adapted to severe environmental conditions, including high altitude and harsh climatic conditions [25]. The Morkaraman breed takes an essential place as a domestic gene source due to its vicinity to the Fertile Crescent region, the first place of domestication with fewer selection studies. The work aimed to investigate the genotypic structures of the myostatin *(MSTN)* gene locus *Hae*III polymorphism and research the relationships between *MSTN* genotypes and a number of traits, e.g., birth weight, weaning weight, weaning age, average daily weight gain, and productivity, in Morkaraman sheep.

Material and Methods

Ethical Approval

The experimental protocol was approved by the Republic of Turkey Ministry of Agriculture Faculty Local Ethics Committee (AEC approval number: 3/2021).

Sampling and DNA Isolation

In the current research, blood samples were collected from unrelated 110 Morkaraman sheep raised as recorded in the pedigree and growth data in the Food and Livestock Application and Research Center (GHUAM), Sheep Breeding Branch at Ataturk University, Erzurum province. DNA was isolated from blood samples by utilizing the Qiagen genomic DNA purification kit.

Polymerase Chain Reaction (PCR)

The PCR reaction was carried out in a final reaction volume of 20 µL, including 4 µL of 10x buffer, 1 µL of every primer (10 pmol/µL, 1 µL$MgCI_2$, 0.5 µLDNTPs, 2.4 µL Taq DNA polymerase, 2 µL of total DNA (50-100 ng), and finally added ultrapure water until reaching a total volume of 20 µL. The amplification of a 337 bp fragment for exon 3 of the sheep *MSTN* locus was performed using the primer pairs reported by Smith et al.[26], forward primer 5'-CCG GAG AGA CTT TGG GCT TGA-3' and reverse primer 5'-TCA TGA GCA CCC ACA GCG GTC-3', with a thermal cycler. For polymerase activation, at the beginning of the PCR cycle, an incubation step was carried out at 95°C for 5 min, followed by 1 cycle of 95°C for 45 s, annealing at 59°C for 45 s, and an extraction step at 72°C for 45 s, followed by 35 cycles of 5 min at 72°C as a final extraction.

Genotyping of the *MSTN* Gene

The restriction endonuclease enzyme *Hae*III digested PCR products of the amplified *MSTN* gene. The incubation of the PCR products was carried out at a temperature of 37°C for a period of 10-12 h in a final volume of 20 µL, containing 8-10 µL of the PCR product, 5 µL of the buffer R, 2.5 µL of the buffer tango, and 6 U *Hae*III restriction enzyme. The digested products were run on 2% agarose gel stained with

EtBr (500 µL/mL in H_2O). Afterward, the digested PCR products were obviously envisioned under UV light.

Data Analysis

The allele gene and genotype frequencies and Hardy-Weinberg test for the examined population were computed in the GenAlEx 6.5 software [27].

One hundred ten pure Morkaraman sheep aged 2-4 years and raised in Atatürk University Food and Livestock Application and Research Center were utilized as the animal material of this study. The sheep were held under semi-extensive conditions. The associations between a number of yield traits and genotypic structures of Morkaraman sheep were studied. To this end, primarily their birth weight, weaning weights and weaning ages, and average daily weight gains were determined as the performance traits of sheep. As reproductive traits, annual lamb rates per sheep were determined, and the lambing rates (productivity) in birth for each ewe mated were calculated. Feeding and management practices were administered in an equal manner to all lambs. When analyzing the acquired data, SPSS statistical software (IBM SPSS 25.0 Corp. Inc.) was utilized based on the general linear model. The association analyses separately examined the impact of genotype on birth weight and productivity; the impacts of genotype and birth weight on weaning age, and average daily weight gain; the impacts of genotype, birth weight, and weaning age on weaning weight.

The statistical models below were employed in accordance with the yield traits in the study.

Yijk:µ+ai+(bj)+(ck)+eijkl

Yijk l: Value of any sheep for the considered performance (birth weight, weaning weight, productivity, weaning age and average daily weight gain) traits

µ: population mean

ai: effect of genotype i (i: 1-3; MM: 1, Mm: 2, mm: 3)

(bj): covariate effect of birth weight j (on weaning age and average daily weight gain)

(ck): covariate effect of weaning age k (on weaning weight)

eijkl: marginal error

In the model used, the genotype and the others' (covariate) effects were accepted as constant, while the error was accepted as random.

Results

Table 1 contains the observed and expected genotype results of the *MSTN* gene *Hae*III polymorphism and the Hardy-Weinberg genetic equilibrium test results in Morkaraman sheep.

The genotypes of the MSTN/*Hae*III polymorphism were found as MM, Mm, and mm, and the percentage frequencies were computed as 9%, 69%, and 22%, respectively *(Table 1)*. The m allele frequency was found as 56%, whereas the M allele frequency was found as 44% for the Morkaraman breed. The Hardy-Weinberg genetic equilibrium test demonstrated that the examined population was not in equilibrium with an X^2 test value of 17.37 and a probability of (P<0.001) *(Table 1)*.

Associations between MSTN/*Hae*III genotypes and a number of performance traits, including birth weight, weaning weight, weaning age, productivity, and weight gain, were researched. *Table 2* demonstrates the least squares mean and standard errors of the MSTN/*Hae*III genotypes concerning several yield traits.

Table 1. *Observed and expected genotypes of the MSTN gene and Hardy-Weinberg genetic equilibrium test results in Morkaraman sheep*

Genotype	(%)	Observed	Expected	X^2 Test	P
MM	(9)	10	20.7	17.37	**
Mm	(69)	75	53.6		
mm	(22)	24	34.7		
Gene frequencies: M: 44%, **m:** 56%					

*** P<0.01*

Table 2. *The least squares means and standard errors of MSTN/HaeIII genotypes in terms of some yield traits in Morkaraman sheep*

Genotype	N	Birth Weight (kg)		Weaning Weight (kg)		Weaning Age (Days)		Productivity		Average Daily Weight Gain (kg)	
		Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
MM	10	4.19	0.255	15.79	1.421	56.9^{a*}	4.20	0.849	0.045	0.202^{b*}	0.015
Mm	75	4.43	0.077	14.59	0.401	49.8^{ab}	1.34	0.871	0.030	0.204^{b}	0.006
mm	24	4.42	0.010	14.84	0.635	46.2^{b}	2.05	0.870	0.051	0.229^{a}	0.008
Total	109	4.41	0.062	14.76	0.333	49.7	1.12	0.867	0.024	0.209	0.005

*SE: Standard Error, * P<0.05*

Genotype did not have a significant impact on birth weight (P>0.05). The birth weight averages of the MM, Mm, and mm genotype groups were revealed to be 4.19 kg, 4.43 kg, and 4.42 kg, respectively, whereas the order was Mm>mm>MM. No significant difference was observed between the means.

Whereas the impact of genotype on weaning weight was insignificant (P>0.05), the impact of birth weight and weaning time was significant (P<0.01). Considering at what % the independent variable affected the dependent variable, the impact of genotype on weaning weight was 3%. The impact of weight was computed as 19%, while the impact of weaning time was computed as 50%. Whereas the mean weaning weights in the MM, Mm, and mm genotype groups were found to be 15.79 kg, 14.59 kg, and 14.84 kg, respectively, the order was inverse to birth weight as MM>mm>Mm. No significant difference was revealed between the means of the genotype groups *(Table 2)*.

While the impact of genotype on weaning age was found to be significant (P<0.05), the impact of birth weight was insignificant (P>0.05). The mean weaning age in the MM, Mm, and mm genotypes was determined to be 56.9 months, 49.8 months, and 46.2 months, respectively, and the order was MM>Mm>mm. The weaning age of the mm genotype was revealed to be shorter than that of the sheep with the MM genotype, and the said difference was statistically significant (P<0.05). However, no statistical difference was determined between the sheep with both genotypes and the heterozygous Mm genotype concerning the weaning age mean (*Table 2*).

The impact of genotype on productivity was determined to be insignificant (P>0.05). Whereas the mean productivity of MM, Mm, and mm genotype groups was acquired as 0.849, 0.871, and 0.870 kg, respectively, the order was Mm>mm>MM. The means of the genotype groups did not differ significantly *(Table 2)*.

The impact of genotype on the mean weight gain from birth to weaning was determined to be significant (P<0.05), while the influence of birth weight was insignificant (P>0.05). Whereas the mean weight gain in the MM, Mm, and mm genotype groups was acquired as 0.202 kg, 0.204 kg, and 0.229 kg, respectively, the order was mm>Mm>MM. The mean weight gain values of the mm genotype were revealed to be statistically significantly (P<0.05) higher compared to those of the sheep with the MM and Mm genotypes *(Table 2)*.

In this study, the polymorphic site of the *MSTN* gene exon 3 region was not associated with birth and weaning weight and productivity. However, there was an association with weaning age and mean daily weight gain, which may be caused by a breed-specific effect.

Discussion

The myostatin gene polymorphisms have been stated to differ in sheep. Soufy et al.[28] observed all of the three genotypes in Sanjabi sheep, while Bayraktar [29] indicated two genotypes, mm and MM, in Iraqi Avassi sheep, and others reported two genotypes, mm and Mm, in Kordi sheep [30], Kalehkoohi sheep [31], Farahani sheep [32], Mehraban sheep [33], and Teleorman Black Head sheep [34]. The studies on polymorphisms reported the higher frequency of the polymorphic *MSTN* gene m allele compared to the M allele gene frequency, and it was found to be consistent with the present research. However, a number of researchers indicated the absence of *MSTN* gene polymorphisms and reported that the mm genotype was monomorphic in Dalagh sheep [35], Zell sheep [36], and Bulgarian sheep [37].

Although some authors reported the Hardy-Weinberg genetic equilibrium in the populations of Mehraban sheep [33], Teleorman Black Head sheep [34], and Iraqi Avassi sheep [29], other authors reported the absence of the Hardy-Weinberg genetic equilibrium in Sanjabi sheep [28], Dalagh sheep [35], Zell sheep [36], and Mehraban sheep [33], which is consistent with the present study results. The above-mentioned discrepancy can be explained by environmental factors, breed differences, population and sampling size, mating strategies, geographical position effect, and genotypic distribution of genetic variants. This result can also be explained, especially by the imbalance caused by selection and migration. The examined population displayed a high degree of genotypic variability for the *MSTN* gene, which may be associated with the breeding plan applied.

Despite genotyping studies on MSTN/*Hae*III polymorphisms, a very low number of studies have been performed on the relationship between genotypes and yield traits. Nevertheless, several findings on the relationship between polymorphic exon 3 of the *MSTN* gene and growth traits have been indicated for weaning weight, 6-month weight, and some carcass traits in Batur sheep, and they have an insignificant effect [11]. Some research has been done on the other polymorphic regions of the *MSTN* gene. In case of the non-expression of myostatin, negative growth regulation fails, and an increase in the number of muscle fibers occurs, resulting in hyperplasia. Boman et al.[3] stated lower daily gain and weaning weight and higher carcass weight in homozygous c.960delG (AA) animals. Furthermore, the AG and GG genotypes led to significant (P<0.001) impacts as more meat and less fatty animals. Nevertheless, mutations in the *MSTN* gene exon 3 influence conformation and adiposity in NWS lambs, causing a carcass with less fat and more muscle mass. An insignificant effect of a mutation in *MSTN* exon 1 and intron 1 regions was revealed on birth, weaning

(3-month), and 6-month weight in Mecheri, Madras Red, and Nilagiri sheep in India [38]. In another PCR-SSCP study, genetic variants were found to be related to meat traits in the myostatin gene, whereas the *MSTN* gene was characterized by decreased total lean meat and increased waist meat ratio [4].

As a result, three genotypes, MM, *mm,* and *Mm*, were determined in the current research at a rate of 9%, 69%, and 22%, respectively. The *M* allele frequency was found to be 44%, whereas the *m* allele frequency was revealed to be 56%. There was a statistical association between the impact of the MSTN/*Hae*III polymorphism and weaning age and average daily weight gain. Nevertheless, no relationship was determined with birth weight, weaning weight, and productivity. The *MSTN* gene exhibits polymorphisms in Morkaraman sheep. However, for it to be regarded as an important genetic marker, further investigations on the *MSTN* gene polymorphisms are suggested in other sheep breeds all around the world to assess potential sheep breeds and use it as a genetic marker in improving growth traits.

Availability of Data and Materials

Data sets are not deposited in different repositories, and data from a third party were not used. The data are original, and users can get it from corresponding author (M. Özdemir).

Ethical Approval

The experimental protocol was approved by the Republic of Turkey Ministry of Agriculture Faculty Local Ethics Committee (AEC approval number: 3/2021).

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Competing Interest

All the authors declare that they have no conflict of interest.

Author's Contributions

Experimental design was conceived by MÖ and NE. ES and KE performed the laboratory experiments, and DT, UDT and SK collected the all data and arranged for the analysis. MÖ performed the statistical analysis and wrote the manuscript. All authors were ranked based on the contribution rates for performing lab studies, collecting the data and literature search and the corrections.

References

1. Bayram D, Akyüz B, Arslan K, Özdemir F, Aksel EG, Çınar MU: *DGAT1, CAST* and *IGF-I* gene polymorphisms in Akkaraman lambs and their effects on live weights up to weaning age. *Kafkas Univ Vet Fak Derg,* 25 (1): 9-15, 2019. DOI: 10.9775/kvfd.2018.20055

2. Ozdemir M, Sönmez Z, Aksakal V: Associations between *Prl/RsaI* polymorphism and some performance traits in Holstein cattle reared under organic condition. *J Anim Plant Sci,* 31 (3): 900-905, 2021. DOI: 10.36899/JAPS.2021.3.0279

3. Boman IA, Klemetsdal G, Nafstad O, Blichfeldt T, Vage DI: Impact of two myostatin *(MSTN)* mutations on weight gain and lamb carcass classification in Norwegian White Sheep *(Ovis aries). Genet Sel Evol,* 42, 4-10, 2010. DOI: 10.1186/1297-9686-42-4

4. Hickford JGH, Forrest RH, Zhou H, Fang Q, Han J, Frampton CM, Horrell AL: Polymorphisms in the ovine myostatin gene *(MSTN)* and their association with growth and carcass traits in New Zealand Romney sheep. *Anim Genet,* 41, 64-72, 2010. DOI: 10.1111/j.1365-2052.2009.01965.x

5. Saleem AH, Ali A, Asif AR, Ali A, Mustafa H, Rashid D, Zafar M, Awais M, Ahmad HI, Baqar Z: Single nucleotide polymorphism and phylogenetic analysis of the exon 2 of *leptin* gene in Lohi sheep. *Adv Life Sci,* 7 (2): 62-65, 2020.

6. Cobanoglu O, Kul E, Abaci SH, Gurcan EK, Cankaya S: Genetic polymorphism of *STAT1* and *STAT5A* genes in Holstein, Jersey, and indigenous cattle breeds in Turkey. *Kafkas Univ Vet Fak Derg,* 26 (2): 255-262, 2020. DOI: 10.9775/kvfd.2019.22908

7. Sonmez Z, Ozdemir M: Determination of polymorphisms in the *GDF5* and *EPS8* genes by HRM analysis in holstein cattle, *Pak J Zoo,* 53 (5): 1857-1865, 2021. DOI: 10.17582/journal.pjz/20200615130606

8. Fındık I, Ozdemir M: Genetic polymorphism of *Pit-1* and *CSN3* genes in Holstein calves and its associations with calf birth weight. *Arch Anim Breed,* 65, 285-292, 2022. DOI: 10.5194/aab-65-285-2022

9. Jia J, Xie D, Zhang Y, Zhang H, Zhang L, Hou S, Chen Q: Association of *BMPR-1B* gene 3'-UTR region polymorphism with litter size in Tibetan sheep. *Kafkas Univ Vet Fak Derg,* 27 (3): 285-289, 2021. DOI: 10.9775/kvfd.2020.25064

10. Uzabaci E, Dincel D: Associations between c.2832A > G polymorphism of *CAST* gene and meat tenderness in cattle: A meta-analysis. *Kafkas Univ Vet Fak Derg,* 28 (5): 613-620, 2022. DOI: 10.9775/kvfd.2022.27770

11. Haren HIH, Purwantini D, Sumaryadi MY, Prayitno P: Polymorphism at third exon of the myostatin gene and its association with growth and carcass traits in Batur sheep. *Biodiversitas*, 21, 2074-2078, 2020. DOI: 10.13057/biodiv/d210534

12. Zhou H, Hickford JGH, Fang Q: Variation in the coding region of the myostatin *(GDF8)* gene in sheep. *Mol Cell Probes,* 22, 67-68, 2008. DOI: 10.1016/j.mcp.2007.08.004

13. Han J, Forrest RH, Sedcole JR, Hickford JGH: Myostatin *(MSTN)* gene haplotypes and their association with growth and carcass traits in New Zealand Romney lambs. *Small Ruminant Res,* 127, 8-19, 2015. DOI: 10.1016/j.smallrumres.2015.03.015

14. Ensembl: *Ovis aries* myostatin *(MSTN)*, mRNA. https://jul2022.archive.ensembl.org/Ovis_aries_rambouillet/Gene/Summary?db=core;g=ENSOARG00020009466;r=2:129059547-129066303;t=ENSOART00020014589; *Accessed:* 5.02.2021.

15. McPherron AC, Lee SJ: Double muscling in cattle due to mutations in the myostatin gene. *Proc Nat Acad Sci USA*, 94, 12457-12461, 1997. DOI: 10.1073/pnas.94.23.12457

16. Broad TE, Glass BC, Greer GJ, Robertson TM, Bain WE, Lord EA, McEwan JC: Search for a locus near to myostatin that increases muscling in Texel sheep in New Zealand. *Proc New Zeal Soc An,* 60, 110-112, 2000. DOI: 10.13140/2.1.4980.6721

17. Marcq F, Larzul C, Marot V, Bouix J, Eychenne F, Laville E, Georges M, Bibe B, Leroy PL, Elsen JM: Preliminary results of a whole genome scan targeting QTL for carcass traits in a Texel Romanov intercross. *Proceedings of the 7th World Congress on Applied Livestock Production,* 19-23 August, Montpellier, France, 2002.

18. Johnson PL, Dodds KG, Bain WE, Greer GJ, McLean NJ, McLaren RJ, Galloway SM, van Stijn TC, McEwan JC: Investigations into the GDF8 *g+6273G-A* polymorphism in New Zealand Texel sheep. *J Anim Sci,* 87, 1856-1864, 2009. DOI: 10.2527/jas.2008-1508

19. Walling GA, Visscher PM, Wilson AD, McTeir BL, Simm G, Bishop SC: Mapping of quantitative trait loci for growth and carcass traits in commercial sheep populations. *J Anim Sci,* 82, 2234-2245, 2004. DOI: 10.2527/2004.8282234x

20. McRae AF, Bishop SC, Walling GA, Wilson AD, Visscher PM: Mapping of multiple quantitative trait loci for growth and carcass traits in a complex commercial sheep pedigree. *Anim Sci*, 80, 135-141, 2005. DOI: 10.1079/ASC41040135

21. Ren H, Li L, Su H, Xu L, Wei C, Zhang L, Li H, Liu W, Du L: Histological and transcriptome-wide level characteristics of fetal myofiber hyperplasia during the second half of gestation in Texel and Ujumqin sheep. *BMC Genom*, 12:411, 2011. DOI: 10.1186/1471-2164-12-411

22. Ansary M, Tahmoorespour M, Valeh MV, Nassiry MR, Shahroudi FE: Investigation of polymorphism of *GDF-8* gene and its association with average daily gain in Baluchi sheep. *Proceedings of the 3rd Congress on Animal Science*, Ferdowsi University of Mashhad, Mashhad, Iran, 2008.

23. Wang J, Zhou H, Hu J, Li S, Luo Y, Hickford JGH: Two single nucleotide polymorphisms in the promoter of the ovine myostatin gene (*MSTN*) and their effect on growth and carcass muscle traits in New Zealand Romney sheep. *J Anim Breed Genet*, 133 (3): 219-226, 2016. DOI: 10.1111/jbg.12171

24. Xing HJ, Wang ZY, Zhong BS, Ying SJ, Nie HT, Zhou ZR, Fan YX, Wang F: Effects of different dietary intake on mRNA levels of *MSTN*, *IGF-I*, and *IGF-II* in the skeletal muscle of Dorper and Hu sheep hybrid F1 rams. *Genet Mol Res,* 13 (3): 5258-5268, 2014. DOI: 10.4238/2014.July.24.4

25. Turkyılmaz D, Esenbuga N: Increasing the productivity of Morkaraman sheep through crossbreeding with prolific Romanov sheep under semi-intensive production systems. *S Afr J Anim Sci*, 49 (1): 185-191, 2019. DOI: 10.4314/sajas.v49i1.21

26. Smith TPL, Lopez-Corrales NL, Kappes SM, Sonstegard TS: Myostatin maps to the interval containing the bovine mh locus. *Mamm Genome,* 8, 742-744, 1997. DOI: 10.1007/s003359900557

27. Peakall R, Smouse PE: GenAlEx 6.5: Genetic analysis in Excel. Population genetic software for teaching and research-An update. *Bioinformatics,* 28, 2537-2539, 2012. DOI: 10.1093/bioinformatics/bts460

28. Soufy B, Mohammadabadi M, Shojaeyan K, Baghizadeh A, Ferasaty S, Askari N, Dayani O: Evaluation of myostatin gene polymorphism in Sanjabi sheep by PCR-RFLP method. *J Anim Sci Res*, 19 (1): 81-89, 2009.

29. Bayraktar M: Determination of *MSTN* gene polymorphism in the Iraqi Awassi sheep. *Int Res J Eng Tech*, 7 (10): 1918-1920, 2020.

30. Akbari S, Dashab G, Alipanah M, Rokouei M, Saghi D: Study on the polymorphism in myostatin gene and its association with growth traits in Kordi sheep of Northern Khorasan. *Modern Genet J*, 10, 129-133, 2015.

31. Ebrahimi A, Rafiei F, Nanakrani S: Genetic polymorphism of *MSTN* gene in kalehkoohi sheep by pcr-rflp method. *The 1st International Conference on New Ideas in Agriculture*, 26 January, Isfahan, Iran, 2014.

32. Shariatzadeh M, Ghazi A, Motlagh MK, Mahdiyeh M: Evaluation of myostatin gene polymorphism in Farahani sheep by PCR-RFLP method. *J Cell Tissue,* 5, 157-163, 2014.

33. Jamshidi S, Karani S, Goudarzi M: Study polymorphism myostatin gene in Mehraban's sheep using PCR-RFLP method. *Sci Int (Lahore),* 26, 1129-1135, 2014.

34. Lazar C, Gras AM, Rotar MC, Pistol GC, Pelmus RS, Ghita E: Identification of myostatin gene polymorphism using PCR-RFLP for improving carcass meat evaluation of Teleorman Black Head lambs. *Sci Pap Anim Sci and Biotech,* 49, 63-68, 2016.

35. Azari MA, Dehnavi E, Yousefi S, Shahmohamadi L: Polymorphism of calpastatin, calpain and myostatin genes in native Dalagh sheep in Iran. *Slovak J Anim Sci,* 12, 1-6, 2012.

36. Dehnavi E, Azari MA, Hasani S, Nassiry MR, Mohajer M, Ahmadi AK, Shahmohamadi L, Yousefi S: Polymorphism of myostatin gene in intron 1 and 2 and exon 3, and their associations with yearling weight, using PCR-RFLP and PCR-SSCP techniques in Zel Sheep. *Biotechnol Res Int,* 2012:472307, 2012. DOI: 10.1155/2012/472307

37. Georgieva S, Hristova D, Dimitrova I, Stancheva N, Bozhilova-Sakova M: Molecular analysis of ovine calpastatin *(CAST)* and myostatin *(MSTN)* genes in Synthetic Population Bulgarian Milk sheep using PCR-RFLP. *J BioSci Biotechnol,* 4 (1): 95-99, 2015.

38. Sahu AR, Jeichitra V, Rajendran R, Raja A: Polymorphism in exon 3 of myostatin *(MSTN)* gene and its association with growth traits in Indian sheep breeds. *Small Ruminant Res*,149, 81-84, 2017. DOI: 10.1016/j.smallrumres.2017.01.009