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The Relationship Between Leptin Gene Polymorphism and Milk Yield Traits in Simmental and Brown Swiss Cattle

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ABSTRACT

This study was performed to determine the genotype and allele frequencies and the association between the leptin gene Sau3AI polymorphism and some performance traits in Simmental (n=60) and Brown Swiss (n=62) cattle in the province of Erzurum, Türkiye. Considering the allele frequencies in the population, the frequency of the A allele was 0.87 and the frequency of the B allele was 0.13 in Simmental cattle, and the frequency of the A allele was 0.94 and the frequency of the B allele was 0.06 in Brown Swiss cattle. According to the analysis conducted in the Simmental breed, the general averages were found to be 5422.4 ± 1901.74 kg for actual milk yield, 5626.6 ± 1475.85 kg for 305-day milk yield, 298.7 ± 84.80 days for lactation duration, and 18.5 ± 4.84 kg for daily milk yield. As a result of the analysis in the Brown Swiss breed, the general averages were 3917.8 ± 1584.38 kg for actual milk yield, 4614.3 ± 982.62 kg for 305-day milk yield, 254.9 ± 99.88 days for lactation duration, and 16.0 ± 3.82 kg for daily milk yield. According to the statistical analysis results, the impact of genotype on milk yield traits was insignificant in Simmental and Brown Swiss cattle.

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Introduction

The livestock sector, with an important role in meeting the animal-based nutrient needs of humans, develops rapidly under the influence of technology and other factors. In parallel with technological advancements, new breeding methods are applied to meet the demand for animal products because of population growth. Two factors affect the phenotypic value of animals in terms of any trait. The first is the genotype, and the other is the environment (Akman, 2016). While determining the quantitative traits of animals in the livestock sector, it is impossible to determine genotypes with ideal alleles by considering only phenotypes since phenotypic values do not always reflect genotypic values. It is only possible to create the superior phenotype in animals by identifying good and efficient genes that affect the character, combining the desired genes in the genotype, and benefiting from the interaction between genes.

The leptin gene takes place among the candidate genes for marker-assisted selection. The leptin gene is expressed in various tissues, including adipose tissue, placenta, mammary glands, skeletal muscles, gastric mucosa, brain, and pituitary glands. Leptin takes a major part in coordinating whole-body energy metabolism and can be classified as a metabolism modifier (Houseknecht et al., 1998). In cattle, the leptin gene is located on the 4th chromosome and consists of three exons (Pomp et al., 1997). Leptin is a 16 kDa protein synthesized by adipose tissue and is involved in regulating feed intake, energy balance, fertility, and immune functions (Fruhbeck et al., 1998).

There is an association between the leptin gene and single-nucleotide polymorphisms and numerous quantitative traits such as carcass fat, feed consumption, and milk yield (Kök et al., 2015; Al-Janabi et al., 2018; Kiyici et al., 2019). Polymorphisms in the leptin gene have been associated with milk performance (Liefers et al., 2002; Heravi et al., 2006; Kiyici et al., 2019), increased perinatal mortality in dairy products (Brickell et al., 2010), calf birth and weaning weights in beef and dairy products (Almeida et al., 2003; Nkrumah et al., 2005; DeVuyst et

al., 2008; Fernandes et al., 2020), and reproductive traits in dairy cattle, such as gestational age (Komisarek and Antkowiak, 2007).

Leptin is a protein with an important role in numerous functions in animals. In addition to its role in the metabolism and growth of animals, leptin has important duties in feed utilization, energy metabolism, and reproductive efficiency. The leptin gene, which plays an important role in all of the above-mentioned economically important mechanisms, is an excellent candidate gene for studies on polymorphism and the association of these polymorphisms with economic traits (Ninov et al., 2008; Özdemir, 2011). Therefore, the objective of the current work was to research the genotypic structures of the leptin gene Sau3AI (LEP/Sau3AI) polymorphism, determine the genotype and allele frequencies in cattle, and associate these genotypes with some performance traits in Simmental and Brown Swiss cattle in Türkiye.

Material and Methods

The animal material of this research consisted of genomic DNA samples acquired from the blood of 62 heads of Brown Swiss cattle and 60 heads of Simmental cattle, raised on two farms carrying out intensive production activities in Erzurum.

The leptin gene specific primers (5'-TCT TAA GCT AGT CAG GTT CCA CAA GGT-3' and 5'-TGC TCC ACG CAG GTG AGC AAG-3'), designed by Ozdemir (2011), were used to amplify a 495 bp fragment in cattle. Amplification reactions were performed in a final volume of 25 µl containing approximately 3 μl genomic DNA, 1 µl dNTP (D7595: Sigma, St. Louis, MO, USA), 0.5 U of Taq DNA Polymerase (D1806: Sigma, St. Louis, MO, USA), 1 µM of each primer, 3 µl of 10x PCR buffer (100 mM Tris-HCl, pH 8.3, 500 mM KCl, 15 mM MgCl2, and 0.01% gelatin), 1 µl of 25 mM MgCl2 and ddH2O. After adding 10 μl of mineral oil to the tubes, PCR amplifications were performed at 94°C for 2 min, 30 cycles of 45 s at 94, 60, and 72°C, which was followed by a final extension at 72°C for 5 min. After the PCR procedure, 8 µl of each PCR product was run on a 1.2% agarose gel at 80 V for 25 min to determine whether amplification occurred. Products with the completed amplifications were stored at -20°C until the next step. To genotype animals for the RFLP, a 7-9 µl PCR reaction mix was used for Sau3A1 enzyme digestion, which was performed in a volume of 20 µl in 0.2 ml sterilized Eppendorf tubes and incubated at 37 ℃ for 12 h. Each 20 µl digestion mix was electrophoresed in a 2.5% agarose gel at 30 V for 2.5 h, and DNA was visualized by staining with ethidium bromide under UV light. A standard DNA marker (P1473: Sigma, St. Louis, MO, USA) was used. The digested AA PCR product exhibited two fragments of 299 and 196 bp. The BB genotype exhibited three fragments of 214, 196, and 85 bp (Table 1). For each cattle breed, leptin allele frequencies were determined by gene counting. The Chi-square (X^2) test was conducted to check whether the populations were in Hardy-Weinberg equilibrium.

In the statistical analysis, the yield records of 60 heads of Simmental cattle and 62 heads of Brown Swiss cattle raised in private enterprises in Erzurum province were used, and milk yield traits such as actual milk yield, 305-day milk yield, lactation period, and daily milk yield were examined as performance traits. Intermittent environmental factors, such as genotype, lactation order, and calving season, which affect the said yield traits, were emphasized.

According to the yield traits in the study, the statistical model below was used.

$$Y_{ijkl}: \mu+a_i+b_j+c_k+e_{ijkl}$$

Y_{ijkl}: The value of any Simmental or Brown Swiss cow in terms of the performance (actual milk yield, 305-day milk yield, lactation period, and daily milk yield) traits considered,
µ: Population mean
$a_{i:}$ effect of genotype i (i: 3; 1: AA; 2: AB; 3: BB)
b_j: effect of lactation order j (j: 2-7),
c_k: effect of calving season k (k: 2; 1: winter and spring, 2: summer and autumn),
$e_{ijkl:}$ marginal error l.

In the model, all the factors, except for the error, were considered constant, while the error was accepted as randomized.

Results and Discussion

DNA samples obtained from the blood of Simmental and Brown Swiss cattle were carried out on a 1.2% agarose gel by PCR, and PCR products were obtained. The agarose gel image of the PCR products is shown in Figure 1.

DNA samples obtained from Simmental and Brown Swiss cattle were amplified in a PCR device, and as a result of cutting the DNA fragments of a 495 bp long PCR product with the restriction enzyme, 299 and 196 bp fragments were obtained for the AA genotype, 214, 196, and 85 bp fragments were obtained for the BB genotype, and 299, 214, 196, and 85 bp fragments were obtained for the AB genotype. It was determined that they formed 214, 196, and 85 bp long bands.

Table 1 contains the genotypic and allele gene frequencies of the breeds, whereas Table 2 presents the Hardy-Weinberg genetic equilibrium test results.

Considering the allele frequencies in the population, the frequency of the A allele was 0.87 and the frequency of the B allele was 0.13 in Simmental cattle, while the frequency of the A allele was 0.94 and the frequency of the B allele was 0.06 in Brown Swiss cattle (Table 2). The leptin gene AA, AB, and BB genotype frequencies were found to be 78.3%, 16.7%, and 5.0% in Simmental cattle and 88.7%, 9.7%, and 1.6% in Brown Swiss cattle, respectively. In both breeds, AA genotype frequencies were the highest in the population, whereas BB genotypes had the lowest frequency (Table 3).

Considering other similar studies, the allele frequencies reported in the studies by Pomp et al. (1997), Rasor et al. (2002), Javanmard et al. (2004), Leifers et al. (2002; 2003), Javanmard et al. (2005), Javanmard et al. (2010), Kulig et al. (2010), Öztabak et al. (2010), Aytekin, (2011), Al-Janabi et al. (2018), Ferchichi et al. (2018), Maletic et al. (2019), Kiyici et al. (2019; 2020), and Fernandes et al. (2020) were similar to the allele frequencies in our study.

Table 1. Restriction enzyme, cutting region, and fragment size of the genotypes

RE	PCR Product (bp)	Cutting region	Genotype and fragment size (bp)
Sau3AI	495	5′-/GATC-3′	AA: 299/196 AB: 299/214/196/85 BB: 214/196/85

Table 2. Leptin genotype and allele gene frequencies of the breeds

Genotype	Brown Swiss		Simmental	
	n	%	n	%
AA	55	88.7	47	78.3
AB	6	9.7	10	16.7
BB	1	1.6	3	5.0
Allele Gene Frequency (%)	A	B	A	B
	94	6	87	13

Table 3. LEP genotype frequencies and Hardy-Weinberg genetic equilibrium test results

Breed	N	Observed	Estimated	X^2 test	P
		AA/AB/BB	AA/AB/BB		
Brown Swiss	62	55/6/1	54.26/7.48/0.26	2.44	ns
Simmental	60	47/10/3	45.07/13.87/1.07	4.67	*

ns: non-significant (P>0.05), *: P<0.05

Table 4. LEP/Sau3AI genotypes with least squares means and standard errors for milk yield traits

Breed	Genotype	N	Actual Milk Yield (kg)		305-day Milk Yield (kg)		Daily Milk Yield (kg)		Lactation period (days)	
			Mean ± SE		Mean ± SE		Mean ± SE		Mean ± SE	
Brown Swiss	AA	113	3997.8	1625.7	4667.8	982.7	16.2	3.81	256.5	101.5
	AB	11	3076.5	943.7	4144.5	949.5	14.4	4.10	234.8	99.2
	BB	4	3973.2	1338.6	4395.4	1229.2	14.8	2.71	263.8	55.4
Total		128	3917.8	1584.4	4614.3	982.6	16.0	3.82	254.9	99.9
Simmental	AA	70	5455.7	1915.4	5665.9	1462.8	18.6	4.80	298.8	85.5
	AB	12	5563.7	2005.6	5739.8	1583.0	18.9	5.19	295.3	83.2
	BB	3	4079.4	585.7	4258.3	969.1	14.0	3.18	308.3	107.2
Total		85	5422.4	1901.7	5626.6	1475.8	18.5	4.84	298.7	84.8

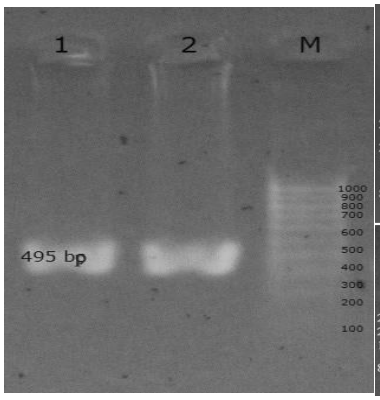

Figure 1. Agarose gel image of PCR products
(M: marker, 1000-100 bp; leptin PCR product: 495 bp)

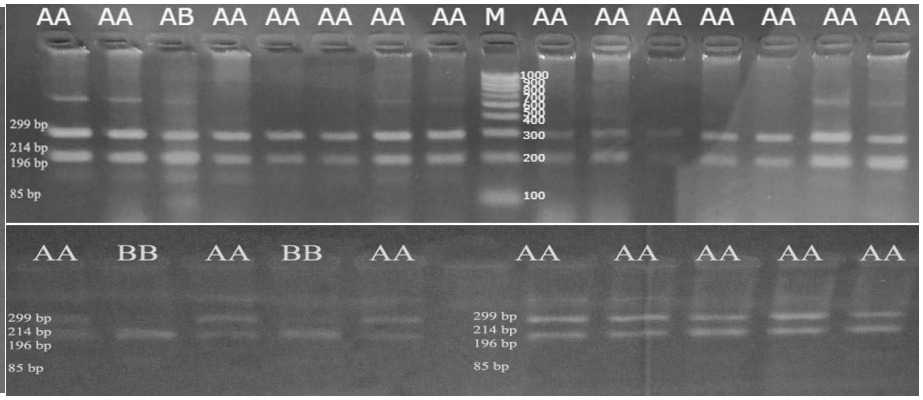

Figure 2. PCR-RFLP gel image of LEP/Sau3AI polymorphism
(M: DNA marker, AA: 299/196 bp, AB: 299/214/196/85 bp, BB: 214/196/85 bp)

According to the Hardy-Weinberg genetic equilibrium test performed for both breeds, LEP/Sau3AI gene polymorphism was in equilibrium in the Brown Swiss breed (X^2=2.44; P>0.05), while it was not in equilibrium in the Simmental breed (X^2=4.67; P<0.05). It is thought that the increase in certain genotypes in the population is prevented or caused by sampling error due to the absence of chance mating in breeding studies.

Table 4 shows the least squares means and standard errors of the leptin gene polymorphic structure in terms of some yield traits in Brown Swiss and Simmental cattle.

Concerning the averages of daily milk yield, actual milk yield, and 305-day milk yields of the LEP/Sau3AI genotype, the highest averages were obtained in the AA genotype in Brown Swiss cattle and the AB genotype in Simmental cattle. The effect of the LEP/Sau3AI genotype on daily milk yield, actual milk yield, and 305-day milk yield was insignificant (P>0.05) in both breeds. The study

determined that cows with the BB genotype had a longer lactation period in both breeds, whereas the effect of the LEP/Sau3AI genotype on the lactation period was insignificant ($P<0.05$) in both breeds. Similar results for milk yield traits have been reported by Madeja et al. (2004) in Polish Black and White bulls, by Gürses (2010) in Jersey, Brown Swiss, Holstein, Eastern Anatolian Red, and Native Black cattle, by Alashawkany et al. (2008) in Holstein cattle, and by Maletic et al. (2019) in Busha cattle. However, studies on Holstein cattle (Moussavi et al., 2006; Kiyici et al., 2019) found that the effect of the LEP/Sau3AI gene on 305-day milk yield was significant. Furthermore, the study on Holstein cows (Liefers et al., 2002) reported that cows with the AB genotype had higher daily milk yield than cows with the AA genotype, and the study on the Brown Swiss breed (Ghazanfari et al., 2006) showed that cows with the AA genotype had higher yield and longer lactation period than cows with the BB genotype.

Conclusion

To determine the LEP gene Sau3AI polymorphism, the genotype and allele frequencies of each cattle breed were determined using the PCR-RFLP method from genomic DNA obtained from Simmental and Brown Swiss cattle. The obtained results were sufficient to reveal the genotype and allele frequencies of the populations, and the correlation analysis demonstrated that the effect of the LEP/Sau3AI polymorphism on the milk yield-related performance traits was insignificant ($P>0.05$) in both breeds. It is thought that demonstrating the usability of such studies in cattle breeding and applying similar studies in different breeds, regions and larger populations will provide significant contributions and new opportunities to developing animal husbandry.

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Author contribution

All authors performed material preparation, data collection, and analysis. Memis Ozdemir prepared the manuscript and wrote the later version.

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Availability of data and material

The data sets generated and/or analyzed during the current study are available from the corresponding author upon reasonable request.

Code availability Not applicable.

Declarations

Ethics Approval

Since ethical approval was obtained in the study of blood samples taken by Gülüzar Sengul (Ataturk University, Faculty of Agriculture, Ethics Committee Head, 2018/1), ethical approval was not required for the current study.

Consent to Participate

Not applicable.

Consent for Publication

Not applicable.

Conflict of Interest

The authors declare no competing interests.

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