

# Lack of evidence for association between the leptin/*Sau3AI* gene and milk yield traits in Holstein Friesian dairy cattle

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## Research Article

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### Abstract

This study aimed to investigate the effect of leptin gene polymorphism and some environmental factors on milk production traits. Blood samples from 212 Holstein Friesian dairy cattle reared on a private farm were used. The intron 2 region of the leptin gene was digested with *Sau3AI* restriction enzyme using the PCR-RFLP method. A and B alleles and AA, AB, and BB genotype frequencies for the *Sau3AI* polymorphism were determined as 0.8821 and 0.1179, and 0.764, 0.236 and 0.000, respectively. Chi-square analysis revealed that the leptin gene polymorphism followed the Hardy–Weinberg equilibrium, including the absence of animals with the BB genotype. The effect of leptin gene polymorphism on all milk production traits was insignificant. For milk production traits, direct heritability ( $h_a^2$ ) varied between  $0.03 \pm 0.283$  (for the dry period) and  $0.50 \pm 0.183$  (for milk conductivity). Regarding the milking time (MT), the estimated breeding values (EBVs) of cattle with the AA genotype were higher than the AB genotype ( $P < 0.05$ ). As a result of this study, in the selection program, allele or genotype could not be suggested as a marker for milk yield characteristics except for the possible exception of milking time and its relationship to mastitis incidence.

Genes with major effects are more important than genes with minor effects in the variation observed among individuals, and such genes are considered candidate genes. One of these genes, the leptin gene, has a pleiotropic effect on the regulation of feed consumption, energy metabolism, body weight, growth, carcass composition, milk and fertility traits, as well as on the immune system in cattle (Taniguchi *et al.*, 2002), and is considered a candidate gene. Several studies have been conducted to determine the association of the leptin gene with yield-related traits in cattle (Liefers *et al.*, 2002; Aytekin, 2011). The associations between *Sau3AI* polymorphism of leptin gene and milk yield (Liefers *et al.*, 2002; Ghazanfari *et al.*, 2006; Metin Kiyici *et al.*, 2019), milk components (Aytekin, 2011; Maletić *et al.*, 2019; Metin Kiyici *et al.*, 2020) and health status (Ferchichi *et al.*, 2018) in various breeds were previously investigated. The sum of the single nucleotide polymorphism variants on which the candidate genes are based (additive genetic effects) results in the breeding value of the animals. However, the high cost of using current molecular analyzes in animal husbandry limits their use or the number of genes examined. We hypothesized that there will be a difference in the population regarding the *Sau3AI* polymorphism of the leptin gene and this difference might impact some milk production traits.

To the best of our knowledge from the literature, there is no study on the relationship between *Sau3AI* polymorphism and milking time (MT), 100 or 200 d of lactation milk yield (LMY<sub>100</sub>, LMY<sub>200</sub>) and dry period (DP) in Holstein Friesian dairy cattle. The aim of this study was to investigate the *Sau3AI* polymorphism of the leptin gene and the effect of this polymorphism and several macro-environmental factors on some milk performance traits in Holstein Friesian dairy cattle.

## Materials and methods

### Animals, sampling and yield recording

Whole blood samples were taken from 212 Holstein Friesian dairy cattle reared on a private dairy farm in Kırşehir province of Türkiye. No ethical approval was required as the samples were taken by a practicing veterinarian for routine management purposes (brucellosis testing) and subsequently made available for the research. Milk production traits obtained from the Afifarm herd management program (Ver 5.4.2) were lactation milk yield as well as average daily, peak, 100, 200 and 305d milk yield (LMY, ADMY, PMY, LMY<sub>100</sub>, LMY<sub>200</sub> and LMY<sub>305</sub>; kg), activity (A; step/day), conductivity (C; mS/cm), milking time (MT; min), days in milk (DIM; day), days to peak milk yield (DPMY; day), and length of dry period (DP;

**Table 1.** Associations between Leptin/Sau3AI gene and milk yield traits in Holstein Friesian dairy cattle

Traits	Genotypes				Regression	Factors	R <sup>2</sup>	
	AA		AB					
	N	$\bar{X} \pm S_{\bar{X}}$	N	$\bar{X} \pm S_{\bar{X}}$				
LMY (kg)	318	9687.84 ± 111	112	9818.36 ± 145	0.380	DIM**, C**, MT**, A*, FCA <sup>NS</sup>	Parity**, calving year**, calving season <sup>NS</sup>	0.6725
A (step/day)	318	170.86 ± 2.35	112	176.24 ± 3.13	0.094	DIM**, MT**, LMY**	Parity*, calving year**, calving season**	0.1492
C (mS/cm)	318	10.15 ± 0.04	112	10.09 ± 0.05	0.313	DIM**, MT <sup>NS</sup> , LMY*	Parity**, calving year**, calving season <sup>NS</sup>	0.3258
MT (min)	318	8.89 ± 0.09	112	8.71 ± 0.12	0.129	DIM**, C <sup>NS</sup> , A**, LMY**	Parity**, calving year**, calving season <sup>NS</sup>	0.3167
LMY <sub>305</sub> (kg)	318	9159.71 ± 114	112	8956.98 ± 155	0.207	FCA*	Parity**, calving year**, calving season <sup>NS</sup>	0.2689
LMY <sub>200</sub> (kg)	318	6794.01 ± 67	112	6777.30 ± 92	0.860	FCA*	Parity**, calving year**, calving season <sup>NS</sup>	0.4105
LMY <sub>100</sub> (kg)	318	3528.29 ± 32	112	3543.29 ± 44	0.739	FCA*	Parity**, calving year**, calving season <sup>NS</sup>	0.5249
DIM (day)	318	328.25 ± 0.83	112	328.61 ± 1.11	0.753	FCA <sup>NS</sup> , SP**, LMY**	Parity**, calving year**, calving season <sup>NS</sup>	0.9726
PMY (kg)	318	48.43 ± 0.41	112	48.08 ± 0.55	0.521	DPMY <sup>NS</sup> , CPMY <sup>NS</sup> , MTPMY*, APMY <sup>NS</sup>	Parity**, calving year**, calving season*	0.5732
DPMY (day)	318	54.53 ± 2.80	112	54.57 ± 3.75	0.990	PMY <sup>NS</sup> , CDPMY <sup>NS</sup> , MTDPMY*, ADPMY <sup>NS</sup>	Parity**, calving year*, calving season <sup>NS</sup>	0.2134
ADMY (kg)	318	29.79 ± 0.34	112	30.13 ± 0.45	0.448	DIM <sup>NS</sup> , C*, MT**, A**, FCA <sup>NS</sup>	Parity**, calving year**, calving season*	0.3894
DP (day)	156	62.32 ± 1.13	62	62.95 ± 1.56	0.675	LMY <sup>NS</sup> , DIM <sup>NS</sup> , CI <sup>NS</sup>	Parity <sup>NS</sup> , calving year**, calving season <sup>NS</sup>	0.1335

NS, non significance; N, number of animals;  $\bar{X}$ , least mean squares;  $S_{\bar{X}}$ , standard error; R<sup>2</sup>, adjusted determination coefficient; LMY, lactation milk yield; A, activity; C, conductivity; MT, milking time; LMY<sub>305</sub>, 305 d of lactation milk yield; LMY<sub>200</sub>, 200 d of lactation milk yield; LMY<sub>100</sub>, 100 d of lactation milk yield; DIM, days in milk; PMY, peak milk yield; DPMY, days to peak milk yield; ADMY, average daily milk yield; DP, dry period; FCA, first calving age; SP, service period; CPMY, conductivity of peak milk yield; MTPMY, milking time of peak milk yield; APMY, activity of peak milk yield; CDPMY, conductivity of days to peak milk yield; MTDPMY, milking time of days to peak milk yield; ADPMY, activity of days to peak milk yield; CI, calving interval.  
\*Statistical significance level (\*P < 0.05, \*\*P < 0.01).

day). Further details as well as details of blood collection, DNA extraction and PCR-RFLP method are specified in the online Supplementary File materials and methods.

### Statistical analysis

The PopGene Version 1.32 software (Yeh *et al.*, 1997) was used for statistical analysis of allele and genotype frequencies and heterozygosity (Nei, 1973) of the gene region. The chi-square ( $\chi^2$ ) test was performed to determine whether the population was in Hardy-Weinberg equilibrium (Düzgüneş *et al.*, 1983). Since the number of animals in the first lactation is high, the analysis of the data is not suitable for the repeated measurement of mixed model, therefore we used the General Linear Model (GLM) to evaluate the effects of genotype and environmental factors. Details of the model are given in the online Supplementary File. Tukey's multiple comparison test was performed to assess the differences between means that were significant as a consequence of the analysis of variance. Minitab v16.1.1 software package (Minitab, 2010) was used for statistical analysis. The multiple trait derivative free restricted maximum likelihood (MTDFREML) package was used to estimate variance components and genetic parameters using the best linear unbiased prediction (BLUP) method (Boldman *et al.*, 1995). Genotype was not

included in the statistical model used to estimate variance components and heritability. Also, factors such as year, parity, and covariance vary in the models according to the different traits. In the estimation of variance components, heritability, and EBVs, the statistically significant factors as a result of the analysis of variance were included in the model (detailed in online Supplementary File and Table S1). The variance components and heritability were estimated using the Restricted Maximum Likelihood (REML) technique in the MTDFREML program. The BLUP technique was used to determine the EBVs in the MTDFREML package program and the breeding value of each animal was calculated as described in the online Supplementary File. A one-way analysis of variance (ANOVA) was performed to examine the variation between genotypes in terms of EBVs. Tukey's multiple comparison test was used to compare the means of genotypes whose effect was found to be significant by analysis of variance.

### Results

#### Sau3AI polymorphism on intron 2 region of leptin gene in Holstein Friesian dairy cattle

The PCR and restriction products of a 422 bp region of the leptin gene region are shown in the online Supplementary Fig. S1. The

**Table 2.** Estimated breeding values of milk yield traits according to genotypes

Traits	Leptin		EBVs $\bar{X} \pm S_x$	Accuracy
	Genotypes	N		
Lactation milk yield (kg)	AA	162	$-21.5 \pm 31.0$	$0.66 \pm 0.05$
	AB	50	$10.2 \pm 55.7$	$0.67 \pm 0.07$
Activity (step/day)	AA	162	$-1.483 \pm 0.782$	$0.69 \pm 0.07$
	AB	50	$0.340 \pm 1.410$	$0.70 \pm 0.08$
Conductivity (mS/cm)	AA	162	$0.013 \pm 0.020$	$0.83 \pm 0.05$
	AB	50	$-0.040 \pm 0.036$	$0.84 \pm 0.05$
Milking time (dk)	AA	162	$0.053 \pm 0.027^a$	$0.72 \pm 0.05$
	AB	50	$-0.068 \pm 0.049^b$	$0.73 \pm 0.06$
305 d of lactation milk yield (kg)	AA	162	$29.3 \pm 35.8$	$0.64 \pm 0.06$
	AB	50	$-77.3 \pm 64.5$	$0.65 \pm 0.07$
200 d of lactation milk yield (kg)	AA	162	$6.2 \pm 27.8$	$0.74 \pm 0.07$
	AB	50	$-19.3 \pm 50.1$	$0.75 \pm 0.08$
100 d of lactation milk yield (kg)	AA	162	$-7.28 \pm 8.67$	$0.67 \pm 0.07$
	AB	50	$-8.00 \pm 15.6$	$0.68 \pm 0.09$
Days in milk (day)	AA	162	$0.102 \pm 0.207$	$0.76 \pm 0.05$
	AB	50	$0.070 \pm 0.373$	$0.77 \pm 0.06$
Peak milk yield (kg)	AA	162	$0.084 \pm 0.142$	$0.69 \pm 0.08$
	AB	50	$-0.142 \pm 0.255$	$0.70 \pm 0.09$
Days to peak milk yield (day)	AA	162	$0.775 \pm 0.607$	$0.56 \pm 0.08$
	AB	50	$0.920 \pm 1.090$	$0.58 \pm 0.10$
Average daily milk yield (kg)	AA	162	$-0.013 \pm 0.112$	$0.78 \pm 0.03$
	AB	50	$0.037 \pm 0.201$	$0.79 \pm 0.04$
Dry period (day)	AA	162	$0.065 \pm 0.087$	$0.59 \pm 0.19$
	AB	50	$0.183 \pm 0.157$	$0.70 \pm 0.17$

N, number of animals; EBVs, estimated breeding values;  $\bar{X}$ , means of EBVs;  $r_{IH}$ , accuracy;  $S_x$ , standard error;  $S_x$ , standard deviation.

<sup>a,b</sup> $P < 0.05$ .

frequencies of A and B alleles and AA, AB, and BB genotypes for the leptin gene polymorphism were determined to be 0.8821 and 0.1179, and 0.764, 0.236 and 0.000, respectively.  $\chi^2$  analysis showed that the leptin gene polymorphism was at the Hardy-Weinberg equilibrium ( $P > 0.05$ ). The results concerning allele and genotype frequencies and heterozygosity values are given in the online Supplementary Table S2.

#### Association analysis, variance components and breeding values

The effects of the *Sau3AI* polymorphism of the leptin gene and some environmental factors on milk production traits as determined by the GLM analysis are shown in Table 1. In addition, the least squares mean and regression coefficients of the environmental factors affecting the milk production traits are detailed in the online Supplementary Tables S3–S6. There were no significant effects of the *Sau3AI* polymorphism of the leptin gene on any of the measured traits (Table 1).

The modeled and estimated variance components and heritability are shown in the online Supplementary Table S7. The  $-2 \log L$  values used to evaluate whether the heritability is proper

for selection program showed variation between  $-1300.13$  (DIM) and  $1483.56$  (DPMY) among all traits. Regarding heritability, it was determined that the direct heritability ( $h_a^2$ ) of different traits varied between  $0.03 \pm 0.283$  (for DP) and  $0.50 \pm 0.183$  (for C), whereas the same figures for maternal heritability ( $h_m^2$ ) were  $0.01 \pm 0.001$  (for LMY<sub>200</sub>) and  $0.25 \pm 0.031$  (for ADMY). For all milk yield traits except LMY<sub>305</sub> and LMY<sub>200</sub>, the correlation between additive genetic effect and maternal effect was found to be high and positive. In production characteristics, it was shown that PMY (63.22%), DP (77.38%) and DPMY (79.94%) were most affected by environmental conditions (online Supplementary Table S7). The estimated breeding values (EBVs) of milk yield features in accordance with the genotype are shown in Table 2. The accuracy of the estimated breeding values (EBV) varied between 56% and 84% when evaluated in terms of genotype. In terms of EBVs, no significant relation between genotypes and milk production traits existed except for MT, for which EBVs of cows with AA and AB genotypes were estimated as  $0.0526 \pm 0.3688$  and  $-0.0682 \pm 0.2593$ , respectively, a significant difference ( $P < 0.05$ ; Table 2). Based on this difference obtained from standardized data, it can be said that the B allele shortens the milking time of the cows in the present study.

## Discussion

In marker-assisted selection research it is critical to investigate the association between candidate genes and yield characteristics in order to improve the effectiveness of selection. There have been numerous studies of leptin gene polymorphism in dairy cattle, and there is no real consensus regarding outcomes. Trakovická *et al.* (2013) reported that the milk yield of Slovak Spotted and Slovak Pinzgau cows with the leptin AA genotype was higher than cattle with the AB and BB genotypes, whereas Al-Janabi *et al.* (2018) determined that the milk yield of Holstein Friesian cattle with the AB genotype was higher than the AA genotype and Maletic *et al.* (2019) stated that the association between milk yield and genotypes in Busha and Busha × Podolian cross-breed cattle was insignificant. Our results differ from the studies of Trakovická *et al.* (2013) and Al-Janabi *et al.* (2018) but are similar to the results of Maletic *et al.* (2019). According to Ferchichi *et al.* (2018), Holstein Friesian cattle with the leptin AB genotype had fewer lameness problems than cattle with the AA and BB genotypes. Unlike the current study, Metin Kiyici *et al.* (2020) noted that conductivity in Holstein Friesian cattle with the BB genotype was significantly higher than in animals with the AA and AB genotypes and Moussavi *et al.* (2006) reported that Holstein Friesian cattle with the AB genotype had a higher LMY<sub>305</sub>. Despite these various differences, Aytekin (2011) claimed that the B allele can be used in selection to increase milk yield due to a statistically non-significant trend between the *Sau3AI* polymorphism and the LMY<sub>305</sub> in Brown Swiss cattle. In Holstein Friesian cows raised in Türkiye, Metin Kiyici *et al.* (2019) observed a significantly lower LMY<sub>305</sub> in leptin BB genotype than either AA or AB (which were similar). Ghazanfari *et al.* (2006) noted that the first 60 and 100 d of milk yield of animals with the AB genotype was higher than with the AA and BB genotypes in Brown Swiss cattle, and suggested that the AA genotype had a more optimal DIM. Al-Janabi *et al.* (2018) found the opposite: DIM in Holstein Friesian cattle with AB genotype was higher than in AA genotypes whilst the AB genotype had an earlier and longer period of PMY than the AA. In an earlier study, Liefers *et al.* (2002) showed that animals with the AB genotype produced more milk (1.23–1.32 kg per day) than the AA genotype, which is different with present study. Under commercial farming conditions, PMY and DPMY vary greatly according to care and feeding, while DP depends on herd management practices, so these differences are perhaps not surprising. Nevertheless, our own findings of lack of significant differences do not agree with any previous reports, but the variation between different studies is noteworthy.

Considering the difficulty of family selection in selection programs for milk yield traits, it was concluded that mass selection would be more appropriate for traits with a heritability of more than 0.25 (A, C, LMY<sub>305</sub>, LMY<sub>200</sub>, DIM, and PMY) while family selection would be more proper for traits with a heritability less than 0.25 (LMY, MT, LMY<sub>100</sub>, ADMY, DPMY, and DP). In a study by Javanmard *et al.* (2010), an insignificant association between the EBVs for Holstein Friesian bulls' milk yield and their leptin genotypes (AA and AB) was determined, and this result agrees with the current study. The only significant association that we observed related to milking time. Vierbauch *et al.* (2021) reported that over-milking caused a 30% change in front teat morphology, but did not cause a significant change in the rear teats. To decrease the risk of mastitis due to MT in Holstein Friesian dairy cattle, the AB genotype could be selected without compromising other milk yield traits.

In conclusion, with the exception of a small effect on milking time, we have failed to identify significant differences in milk yield traits as a consequence of leptin gene polymorphism in Holstein Friesian dairy cattle.

**Supplementary material.** The supplementary material for this article can be found at <https://doi.org/10.1017/S0022029923000717>

**Acknowledgments.** The farm where the study was conducted and completed Ph.D. thesis are also included in the Supplementary File.

## References

- Al-Janabi H, Al-Rawi A and Al-Anbari N (2018) Genetic polymorphisms of leptin gene associated with production traits in Holstein primiparous cows. *Plant Archives* **18**, 2311–2316.
- Aytekin İ (2011) Associations between leptin and Pit-1 gene polymorphisms with milk yield and milk composition traits in Brown Swiss cattle raised in Konkular state farm (Ph. D Thesis). Selçuk University.
- Boldman KG, Kriese LA, Van Vleck LD, Van Tassell CP and Kachman SD (1995) A manual for use of MTDREML: a set of programs to obtain estimates of variances and covariances. *US Department of Agriculture Agricultural Research Service* **114**, 1–116.
- Düzgüneş O, Kesici T and Gürbüz F (1983) İstatistik metodları. Ankara Üniversitesi Ziraat Fakültesi Yayınları. In Turkish.
- Ferchichi MA, Jemmali B, Amiri S, Ben Gara A and Rekiç B (2018) Effect of leptin genetic polymorphism on lameness prevalence in Tunisian Holstein cows. *Archives Animal Breeding* **61**, 305–310.
- Ghazanfari S, Nassiry M and Moussavi AH (2006) Polymorphism in gene leptin and its relationship to milk production and reproduction traits in Brown Swiss cows. *Proceedings of the British Society of Animal Science* **206**, 90.
- Javanmard A, Khaledi K, Asadzadeh N and Solimanifarjam AR (2010) Detection of polymorphisms in the bovine leptin (LEP) gene: association of single nucleotide polymorphism with breeding value of milk traits in Iranian Holstein cattle. *Journal of Molecular Genetics* **2**, 10–14.
- Liefers SC, Te Pas MFW, Veerkamp RF and Van Der Lende T (2002) Associations between leptin gene polymorphisms and production, live weight, energy balance, feed intake, and fertility in Holstein heifers. *Journal of Dairy Science* **85**, 1633–1638.
- Maletić M, Paprikić N, Lazarević M, Hodžić A, Davidović V, Stanišić L and Stanimirović Z (2019) Insight in leptin gene polymorphism and impact on milk traits in autochthonous Busha cattle. *Acta Veterinaria* **69**, 153–163.
- Metin Kiyici J, Arslan K, Akyuz B, Kaliber M, Aksel EG and Çınar MU (2019) Relationships between polymorphisms of growth hormone, leptin and myogenic factor 5 genes with some milk yield traits in Holstein dairy cows. *International Journal of Dairy Technology* **72**, 1–7.
- Metin Kiyici J, Akyüz B, Kaliber M, Arslan K, Aksel EG and Çınar MU (2020) LEP and SCD polymorphisms are associated with milk somatic cell count, electrical conductivity and pH values in Holstein cows. *Animal Biotechnology* **31**, 498–503.
- Minitab (2010) *Minitab 16.1.1. for Windows*. State College, PA, USA: Minitab Inc.
- Moussavi AH, Ahouei M, Nassiry MR and Javadmanesh A (2006) Association of leptin polymorphisms with production and reproduction traits in Iranian Holstein dairy cows. *In Proceedings of the British Society of Animal Science* **2006**, 85–85.
- Nei M (1973) Analysis of gene diversity in subdivided populations. *Proceedings of the National Academy of Sciences* **70**, 3321–3323.
- Taniguchi Y, Itoh T, Yamada T and Sasaki Y (2002) Genomic structure and promoter analysis of the bovine leptin gene. *IUBMB Life* **53**, 131–135.
- Trakovická A, Moravčíková N and Kasarda R (2013) Genetic polymorphisms of leptin and leptin receptor genes in relation with production and reproduction traits in cattle. *Acta Biochimica Polonica* **60**, 783–787.
- Vierbauch T, Peinhopf-Petz W and Wittek T (2021) Effects of milking, over-milking and vacuum levels on front and rear quarter teats in dairy cows. *Journal of Dairy Research* **88**, 396–400.
- Yeh FC, Yang RC, Boyle TB, Ye ZH and Mao JX (1997) POPGENE, the user-friendly shareware for population genetic analysis. *Molecular Biology and Biotechnology Centre University of Alberta Canada* **10**, 295–301.