

EFFECTS OF *DGAT1* AND *GH1* POLYMORPHISM ON MILK YIELD IN HOLSTEIN COWS REARED IN TURKEY

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Summary: The aim of this study was to analyse the associations among the K232A polymorphism in the diacylglycerol acyltransferase1 (*DGAT1*) gene and L127V polymorphism in the bovine growth hormone (*GH1*) gene and milk yield in Holstein cows. A total of 281 Holstein cows from three different dairy cattle farms in the Burdur and Kayseri provinces of Turkey were included in this study. The PCR-RFLP method was used for *DGAT1* and *GH1* genotyping. The frequencies of genotypes and alleles of *GH1* gene were found to be 0.78 for LL, 0.18 for LV and 0.04 for VV; 0.87 for the L allele and 0.13 for the V allele. The frequencies of genotypes and alleles of the *DGAT1* gene were to be 0.61 for AA, 0.30 for KA and 0.09 for KK; 0.76 for the A allele and 0.24 for the K allele. No relations were found between *DGAT1*-K232A genotypes and the average milk yield in the first three lactations. However, an association between *GH1*-L127V polymorphism and the average milk yield of Holstein cows was found in the first three lactations. Cows with LL genotype had higher milk yield compared to LV and VV cows ($P < 0.05$).

Key words: *DGAT1*; *GH1*; Holstein; milk yield; PCR-RFLP

Introduction

Estimation of the future performance of livestock is a crucial subject in farm animal breeding. Male and female animals with superior features should be selected to accelerate genetic improvement (1). The genetic potential of breeder candidates can be directly determined using the available molecular genetic methods (2). For this purpose, quantitative genetic methods have been used. Recent developments in the field of molecular

biology have been used in livestock breeding and selection methods as an additional tool (3).

Selection programmes in dairy cattle breeding are basically aimed at milk production traits, milk components and fertility. These traits are known as polygenic traits, and they are controlled by numerous genetic loci and influenced by many environmental factors. Therefore, it is thought that candidate genes with close linkage of the encoding loci can be used to estimate milk production performance (4). Several polymorphisms in different gene loci have been noted to affect production traits such as milk yield and milk composition (2). For instance, it has been reported that there are

strong association between acylCoA-diacylglycerol acyltransferase1 (*DGAT1*) genotypes and milk traits (5); bovine growth hormone (*GHI*) genotypes and milk yield (6). Many genome scans have shown that QTLs on *Bos taurus* autosomal (BTA) chromosomes 6, 14, 20, and 26 have significant effects on milk yield traits (7). It is thought that the selection of animals with favourable genotypes of genetic markers may be possible, thus allowing rapid genetic progress in dairy cattle breeding. Particularly in the last few decades, with regard to this topic, several potential candidate genes have been recognised in cattle.

Triglyceride synthesis is catalysed by *DGAT1*. This enzyme plays a major role in intestinal fat absorption, lipoprotein synthesis, the development of adipose tissue and lactation in higher eukaryotes (8). The bovine *DGAT1* gene, which is located on the centromeric end of BTA14, has been reported as a candidate gene for QTLs associated with fat content and milk yield (5, 9, 10, 11).

Bovine growth hormone directly or indirectly affects many physiological processes such as lactation, growth and reproduction (12). Therefore, it is thought that *GHI* can be considered as a genetic marker of milk productivity in cattle (13). Previous studies have shown that there might be an association between allelic variants of the *GHI* gene and milk yield traits (14). Several polymorphisms were found in the *GHI* gene (1, 4). The best known of these polymorphisms is the leucine (L allele) to valine (V allele) substitution at position 127 in exon 5 of the *GHI* gene. The effects of L127V polymorphism on milk production in cattle have been studied to some extent but the results obtained by various researchers have been found to be contradictory (1, 15).

The Holstein is the most commonly reared dairy cattle breed (16) and 92% of annual milk production is obtained from imported breeds such as Holstein, Simmental and Brown Swiss and their crossbreeds in Turkey. The association among *GHI* and *DGAT1* gene polymorphisms and milk yield has been studied in different countries (1, 11, 12). However, according to the authors' knowledge, no study has as yet been conducted on the association among *GHI* and *DGAT1* polymorphisms and milk yield in the Holstein breed in Turkey.

The aim of this study was to analyse the association of *GHI* and *DGAT1* gene polymorphisms with average (1., 2. and 3. lactations) milk yield in a Turkish Holstein-Friesian cow population.

Materials and methods

PCR-RFLP assay for DGAT1 and GHI genotypes

A total of 281 Holstein cows, in the third lactation, were used in this study. The DNA was extracted from whole blood using phenol-chloroform method (17).

For detection of *DGAT1* genotypes, a 411 bp DNA fragment was amplified by PCR. PCR was carried out in a 25 µL volume containing 5 pmol of each primer (forward: 5'-GCACCATCCTCTTCTCAAG-3'; reverse: 5'-GGAAGCGCTTTCGGATG-3') (Genbank no. AJ318490.1) 1.5 mM MgCl₂, 200 µM dNTP mix, 1 X PCR buffer, 1U Taq polymerase and 100 ng of genomic DNA template. PCR included the following steps: pre-denaturation of 95 °C/5 min followed by 35 cycles at 94 °C/1 min, 60 °C/1 min, 72 °C/1 min and final extension at 72 °C/10 min. Within the *DGAT1* gene the non-conservative polymorphisms K232A at position 10433 and 10434 in exon 8 giving rise to a lysine by alanine amino acid substitution. The amplified PCR products were digested using the *CfrI* (MBI Fermentas) enzyme. The PCR mixture for the *GHI* gene was prepared in the same way as for *DGAT1*, and a 223 bp DNA fragment was amplified. PCR products were amplified using forward 5'-GCTGCTCCTGAGGGCCCTTCG-3' and reverse 5'-GCGGCGGCACTTCATGACCCT-3' (Genbank no. JQ711182.1) primers. The cycles applied were as follows; pre-denaturation at 95 °C/4 min, followed by 35 cycles at 94°C/40sec, 60 °C/40 sec, 72°C/40 sec and the final extension at 72 °C/5 min. Amplified PCR products were digested with the *AluI* (MBI Fermentas). Polymorphism is the leucine (L allele) to valine (V allele) substitution at position 127 in exon 5 of the *GHI* gene.

Data sets and statistical analysis

Data for daily milk production in the first three lactations were obtained from the Cattle Breeders' Association of Burdur and Kayseri. Direct counting was used to estimate the genotype and allele frequencies of the *DGAT1* gene *CfrI* and *GHI* gene *AluI* genetic variants. The chi-square test (χ^2) was used to check whether the populations were in Hardy-Weinberg equilibrium using PopGene32 software (18). Mean differences of milk yield among genotype groups were assessed by analysis

Table 1: Allelic and genotypic frequencies of *DGAT1* and *GH1* genes

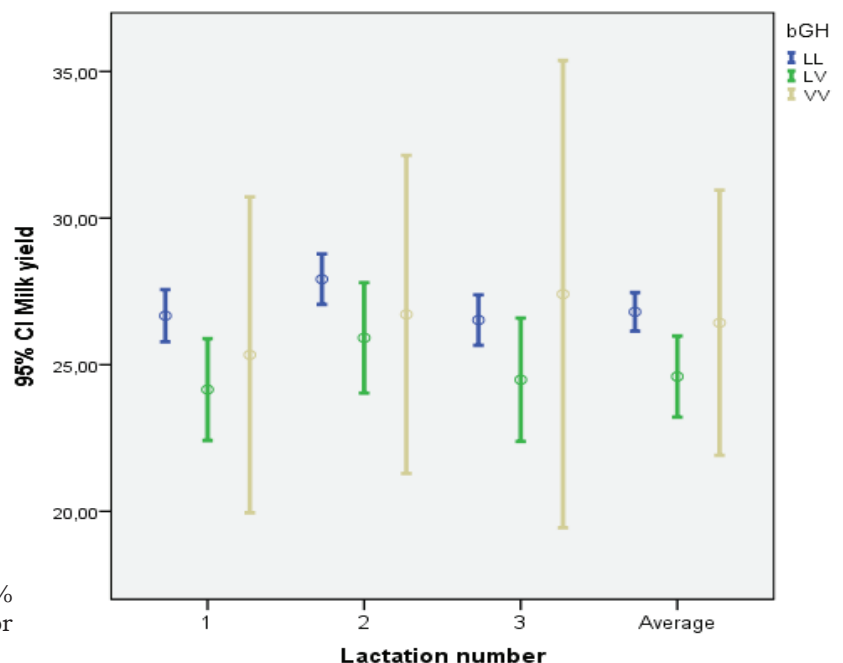
Loci	Frequency	Genotype			Allele Frequency		Statistical Significant (Chi-squared HWE)
		AA	KA	KK	A allele	K allele	
<i>DGAT1</i>	Observed	172	83	26	0.76	0.24	$X^2 = 10.23$ $P < 0.01$ (df=1)
	Expected	162.2	102.6	16.2			
<i>GH1</i>	Observed	219	51	11	0.87	0.13	$X^2 = 10.91$ $P < 0.001$ (df=1)
	Expected	212.7	63.5	4.7			

HWE: Hardy-Weinberg Equilibrium; df: degree of freedom

Table 2: Means and standard error of mean (SEM), coefficient of variation of milk yields (average daily milk yield in the first three lactations) in Holstein cows with different *DGAT1* and *GH1* genotypes

Loci	Genotype	N	Mean \pm SEM (liter)	%V	Statistical Significance (ANOVA)	
<i>DGAT1</i>	AA	172	26.55 \pm 0.39	19.2	F: 1.73 P>0.05	
	KA	83	25.64 \pm 0.55	19.6		
	KK	26	27.60 \pm 0.93	17.2		
		$\beta = 0.20$	$P = 0.966$	$R^2 = 0.001$		
<i>GH1</i>	LL	219	26.79 \pm 0.33 ^a	18.4	F: 3.95 P<0.05	
	LV	51	24.60 \pm 0.69 ^b	20.0		
	VV	11	26.43 \pm 2.03 ^{ab}	25.4		
		$\beta = -1.18$	P = 0.042	$R^2 = 0.015$		

^{a,b,c} : Different superscripts within the same column demonstrate significant differences; %V: Coefficient of variation; β : Regression coefficient; R^2 : Determination coefficient

**Figure 1:** Error Bar graph with 95% Confidence interval (CI) of milk yields for *GH1* genotypes in Holstein cows

of variance and Sidak multiple comparisons test as post hoc test. The effects on the average milk yield of *GH1* and *DGAT1* genotypes were estimated using regression analysis. The software SPSS for Windows Version 14.01 (License number: 986964) was used in statistical analysis data.

Results

PCR amplification yielded a 411 bp long *DGAT1* gene fragment. Restriction digestion of 208 and 203 bp PCR products with the *CfrI* enzyme revealed three genotypes of AA (208 and 203 bp), KK (411 bp) and KA (411, 208 and 203 bp). PCR amplification yielded a 223 bp long *GH1* gene fragment. Restriction digestion of 171 and 52 bp PCR products with the *AluI* enzyme revealed three genotypes of LL (171 and 52 bp), VV (223bp) and LV (223, 171 and 52 bp). The allelic and genotypic frequencies of the *DGAT1* and *GH1* genes, and the polymorphisms for the Holstein cows are given in Table 1. Significant deviation was observed from HWE in the Holstein breeds on *DGAT1* ($P < 0.01$) and *GH1* ($P < 0.001$) genes.

Our findings revealed that no-significant difference was found among cows with different *DGAT1* genotypes (AA, AK, KK) in terms of average milk yield per day ($P > 0.05$). The LL and VV genotypes of the *GH1* gene were better than the LV genotype for average daily milk yield (Table 2) ($P < 0.05$). Additionally it was found that animals who carry the LL genotype had a higher, and more homogeneous milk yield than those with the other two genotypes (Figure 1). On the other hand, it was found that VV genotype cows show a similar feature together with LL and LV genotypes in terms of daily milk yield, but VV genotype cows showed a higher variation coefficient (25%) (Table 2).

In this study, regression analysis was also performed and the regression coefficient values of genotypes were calculated. The effect of *DGAT1* genotypes on average milk yield was not found significant ($p = 0.966$). The regression coefficient (R^2) value of the *DGAT1* gene was found to be very low (< 0.001) in the Holstein cows studied. On the other hand, the effects of *GH1* genotypes on milk yield were found to be significant, and the regression coefficient (R^2) value was found as 0.015.

Discussion

In this study, due to their potential roles in milk yield, the *GH1* and *DGAT1* genes were studied to evaluate their effects on milk yield in Holstein cows.

The frequency of the *GH1*-V allele was found to be lower than that of the L allele; this has been generally reported in all Holstein populations (12, 15). Similarly, in this study, the frequency of the *GH1*-V allele was found to be lower than that of the L allele. It was reported that the highest frequency of the L allele was found in larger sized dairy breeds such as Holstein, and this allele was also correlated with higher milk production (19). In addition, it was reported that selection for milk yield in the German Holstein cattle population has been indirectly effective in the spreading of the *GH1*-L allele. Hence, the frequency of the V allele was found to be very low in German Holstein sires (12). Similarly, the *GH1*-L allele frequency was also found to be higher than that of the V allele in our study. The high *GH1*-L allele frequency in the examined Holstein cattle may have caused the deviation from HWE. On the other hand, it is aimed to increase the milk yield in Holstein breeding in Turkey for many years. Hence, it may lead to HWE in terms of *GH1*-L allele in the Turkish Holstein population. It is thought that this selection in *GH1* gene may cause deviation from HWE in *DGAT1* gene as well. In addition, Thaller et al. (11) reported that *DGAT1*-A allele frequency was found to be higher than that of the K allele and was fixed in German Holstein cattle population. This information may be an explanation of the deviation from HWE for *GH1* and *DGAT1* genes in the Holstein cows examined in our study, because an important part of the Turkish Holstein cattle population originated from Germany and the US (20). Similarly, *DGAT1*-A allele frequency was also found to be considerably higher than that of the K allele in our study. On the other hand, the deviation from HWE for *GH1* and *DGAT1* genes may be due to the number of animal population used in this study.

It was reported that there was a significant association between the *GH1*-L127V genotypes and milk production traits in different cattle breeds (13, 21). Nevertheless, this information is still controversial. For example, Zwierzchowski et al. (22) reported that the presence of the V allele

may be indicative of better performance in daily milk yield and milk composition in Holstein cows. Similarly, V allele frequency was reported to be very common in best Canadian Holstein AI bulls by Sabour and Lin (23). Kovacs et al. (1) found that the V allele was preferred for increased milk production traits in a Hungarian Holstein cattle population. Khatami et al. (13) investigated the relationship between *GH1* genotypes and milk production in a Russian Holstein cattle population and the Yaroslavl cattle breed, and they found that the rate of cows with high milk production (more than 6000 kg per lactation) was higher in cows with the VV genotype than in those with other genotypes. This rate was 1.75 times higher in cows with the VV genotype than in cows with LL and LV genotypes, respectively. The V allele was reported by Khatami et al. (13) to be superior commercially to the L allele, especially when present in homozygote form. On the other hand, there are studies indicating that the LV genotype is superior to other genotypes for milk yield. For instance, in Polish Holstein bulls and heifers, significant differences were reported between *GH1* genotypes by Grochowska et al. (24), and the highest values for milk and protein yields were observed for cows with the LV genotype. Similarly, Kovács et al. (1) reported that the *GH1*-LV genotype compared to other genotypes was shown to have a positive effect on 305 days lactation yield. Furthermore, there have also been studies in which the L allele was associated with high milk yield. For instance, the LL genotype of the *GH1* gene was significantly associated with better milk production traits, mainly with fat content in the Brown Swiss breed (6). Furthermore, cows with the LL genotype were shown to have a higher milk yield than *GH1*-LV cows in the first lactation. No significant differences between the genotypes and milk production traits in the second and third lactations in Polish Holstein cows were found by Dybus (15). In this study, the LL genotype of the *GH1* gene was significantly better for milk yield than the other two genotypes in Turkish Holstein cows.

DGAT1 is another gene which is thought to be associated with milk yield in cattle, and this gene has two alleles. The *DGAT1*-K allele has been associated with high milk fat yield (25), the *DGAT1*-A allele has been associated with high milk yield (5, 11).

The milk fat yield has become a desired characteristic in dairy cattle breeding in recent years. This situation may have been caused by

an increase in *DGAT1*-K allele frequency in some populations. As a result of selection to increase milk yield, the frequency of the *DGAT1*-K allele decrease from 15 to 5% between 1981 and 1990 in Israel Holstein cows (26).

The milk yield of daughters of bulls with *DGAT1*-AA genotypes was found on average to be 548 kg higher than that of bulls with *DGAT1*-KK genotypes in the Holstein breed (12). Similarly, the *DGAT1*-A allele was found to have economically beneficial effects in German Holstein across all lactations. However, it was reported that this allele showed better performance in Fleckvieh cattle in only the first lactation (11).

On the other hand, there have been studies in which no relationship between *DGAT1* genotypes and milk yield existed. For instance, Näslund et al. (27) found no differences among the three *DGAT1* genotypes and milk yield in Swedish Holsteins. Similarly, we did not find any relationship between *DGAT1* alleles and milk yield in Holsteins in our study. To estimate the possible effects of *DGAT1* alleles on milk yield traits more studies should be conducted in dairy cattle breeds.

Milk yield is a multifactorial trait, and milk yield traits have been shown to be primarily and considerably influenced by environmental factors such as farm environment, management and feeding. Therefore, the observed differences between the *GH1* and *DGAT1* genotypes and milk production characteristics could have resulted from another source of variation such as the effects of herd and sire. The effects of the *GH1* and *DGAT1* genotypes on the milk yield, milk fat and milk protein content should be confirmed in further studies. However, it is not yet possible to say which genotypes of *GH1* and *DGAT1* should be recommended for the improvement of milk production traits.

In terms of herd management in farms, high and uniform milk yield is very important. It was found in this study that LL genotype cows show uniformity in terms of daily milk yield, whereas VV genotype cows have a higher variation coefficient. The study showed that *GH1* may be used in selection programmes in dairy cattle breeding. The crucial role of growth hormone in lactation initiation and maintenance is well known, thus *GH1* polymorphisms and interaction with other yield traits should be the subject of further research. Furthermore, *DGAT1* polymorphism was not found to have a significant effect on milk yield.

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VPLIVI POLIMORFIZMA *DGAT1* IN *GH1* NA MLEČNOST KRAV PASME HOLSTEIN, GOJENIH V TURČIJI

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Povzetek: Namen raziskave je bil proučiti povezave med polimorfizmom K232A v genu za diacilglicerol acyltransferaso 1 (*DGAT1*) in polimorfizmom L127V v genu govejega rastnega hormona (*GH1*) ter mlečnostjo krav holstein. Skupno je bilo v raziskavo vključenih 281 privesnic in krav pasme holstein v drugi laktaciji s treh različnih mlečnih govedorejskih kmetij v provincah Burdur in Kayseri v Turčiji. Za genotipizacijo *DGAT1* in *GH1* je bila uporabljena metoda PCR-RFLP. Frekvence genotipov in alelov gena *GH1* so bile 0,78 za LL, 0,18 za LV, 0,04 za VV, 0,87 za alel L in 0,13 za alel V. Frekvence genotipov in alelov gena *DGAT1* so bile 0,61 za AA, 0,30 za KA, 0,09 za KK, 0,76 za alel A in 0,24 za alel K. Med genotipi *DGAT1*-K232A in povprečno mlečnostjo v prvih treh laktacijah niso bile ugotovljene povezave. Ugotovljena pa je bila povezava med polimorfizmom *GH1*-L127V in povprečno mlečnostjo krav pasme Holstein v prvih treh laktacijah. Pri kravah z genotipom LL je namreč mlečnost višja kot pri kravah z genotipoma NN in VV ($p < 0,05$).

Ključne besede: *DGAT1*; *GH1*; holstein; proizvodnja mleka; PCR