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RESEARCH ARTICLE

Comparision of DGAT1 K232A Polymorphism and its Effects on some Milk Quality Parameters in Holstein and Native Black Race Cattles [1]

Harun CERİT [1] Emek DÜMEN [2] Funda Hatice SEZGİN [3]

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[1] İstanbul University, School of Veterinary Medicine, Department of Animal Breeding and Genetics, TR-34320 Avcılar, İstanbul - TURKEY
[2] İstanbul University, School of Veterinary Medicine, Department of Food Hygiene and Technology, TR-34320 Avcılar, İstanbul - TURKEY
[3] İstanbul University, School of Engineering, Department of Industrial Engineering, TR-34320 Avcılar, İstanbul - TURKEY

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Summary

In this study, effects of DGAT1 K232 polymorphism and the allele frequency differences of "K" (alanine variant) and "L" (lysine variant) on some cattle milk qualities and some microbiological parameters which pose a risk on consumer health were investigated. For this purpose, 2 years old 50 cattles were used as material (25 Holstein race and 25 Native Black race). Blood samples were collected for determining the allele frequencies of DGAT1 gene. Then, during the experimental period (1 month) the milk of the cattles were collected once a week and the milk samples has been explored for some important foodborne pathogens (Total Aerobic Bacteria, Coliforms, *Escherichia coli*, *Listeria monocytogenes* and *Staphylococcus aureus*) and milk quality parameters (pH, fat, density and acidity). According to the findings, it has been identified that DGAT1 gene frequency differences were significantly effective on some the parameters of milk qualities and the growth of coliforms, *Esherichia coli* and *Staphylococcus aureus*.

Keywords: *DGAT1 gene, Milk quality, Polymorphism, Cattle, Foodborne pathogens*

Holstein ve Yerli Kara Sığır Irklarında DGAT1 K232a Poliformizminin ve Bunların Bazı Süt Kalite Parametrelerine Etkisinin Araştırılması

Özet

Bu çalışmada, farklı ırk sığırlarda DGAT1 K232 polimorfizmi araştırılmış ve DGAT1 geninde bulunan "K" (alanin varyantı) ve "L" (lizin vasryantı) allel frekans farklılıklarının sığırların bazı süt kalite (süt verimi, sütte yağ oranı, pH vb.) parametrelerine ve tüketici sağlığı açısından risk teşkil eden önemli bazı gıda kaynaklı patojenlere etkisi incelenmiştir. Bu amaçla 2 yaşlı, 50 adet sığır (25 adet Holstein ırkı, 25 adet ise Yerlikara ırkı) materyal olarak kullanılmıştır. Sığırlardan kan örnekleri alınarak DGAT1 geni allel frekansları belirlenmiş ve daha sonraki dönemde ise haftada 1 kez olmak üzere 1 ay boyunca aynı sığırlardan süt örnekleri toplanmış ve toplanan süt örnekleri bazı önemli gıda kaynaklı mikrobiyolojik parametreler (toplam mezofilik erobik bakteri, koliformlar, *Escherichia coli, Listeria monocytogenes* ve *Staphylococcus aureus* olmak üzere) ve süt kalite parametreleri (pH, süt yağı, sütün yoğunluğu ve sütün asiditesi olmak üzere) açısından incelenmiştir. Elde edilen bulgulara göre DGAT1 geni allel frekans farklılıklarının, tüm süt kalite parametreleri ve mikrobiyolojik parametrelerden koliformlar, *Esherichia coli* ve *Staphylococcus aureus* üremelerinin üzerine istatistik açıdan belirgin derecede etkili olduğu tespit edilmiştir.

Anahtar sözcükler: *DGAT1 geni, Süt kalitesi, Polimorfizm, Sığır, Gıda kaynaklı patojenler*

INTRODUCTION

DGAT1 gene is determined as a 8.6 kb sized gene including 17 exon territories and its relation to milk productiveness in cattles is proven. According to rat trials, lack of double *DGAT1* gene results in the complete

İletişim (Correspondence)
+90 212 4737070/17154
emekdumen@yahoo.com

prevention of milk secretion due to the lack of triglyceride synthesis in mammary gland. The transformation of lysine amino acid in exon 8 region 232 of *DGAT1* gene to alanine results in two different haplotypes. The haplotype which encodes the lysine amino acid is the principal type, whereas the haplotype that encodes the alanine amino acid is the mutant one [1].

Diacylglycerol O-acyltransferase 1enzyme catalyses the last step of triglyceride synthesis in cellular triglyceride metabolism. The enzyme also carries important functions in adiposis tissue and intestinal fat absorption [1]. In European cattle races, it has been reported that the aforementioned gene may have some polymorphologic properties with the ability of lysine and alanine switching places/lysine replacing alanine in the 10433 and 10434 loci (positions) of the eighth exon (*DGAT1 K232A*) [2]. The milk can have higher rate of fat due to the mutation of "K" allele. Literature may also report that the "K" allele is responsible from the saturated fat acids in the milk [3]. The individual properties of the related loci of the cattle races can affect the *DGAT1 K232A* polymorphism and this may produce different effects on milk fat [4,5]. The utility of the cattles for milk and meat production, their adaptation to natural conditions and tolerance to diseases makes them economically advantageous [6,7]. Due to the mentioned reasons, the investigations of *K232A* polymorphism in cattle plays an important role in in milk industry and procurement of higher quality milk, thus consumer health.

MATERIAL and METHODS

Collection of Blood and Milk Samples

Fiftty cattles that are 2 years old have been used as test subjects of this study (with the acceptance number of 2012/16 by İstanbul University Local Ethical Committe of Animal Experiments). Blood samples have been collected from 25 Holstein race cattles which are thought to have *DGAT1* gene and high alele frequency and 25 indigeneous race cattles (native black), which are thought to have *DGAT1* gene but low allele frequency. Following this, the blood samples have been analyzed in molecular genetic methods and the cattles which have the *DGAT1* gene and which have high and low frequencies of "K" and "A" alleles have been positively identified. After that, the cattles which carry high allele frequency and low allele frequency have been grouped and milk enough for the microbiological and milk quality parameter analyses has been harvested from these test subjects every week for a month. During the study trial period, in order to maximize the homogenization, it has been provided that all the cattles are kept under the same conditions (hygienic conditions, feeding, age etc.).

Determination of DGAT1 Gene

Lysine (K) and the Alanine (A) alleles in the *DGAT1* gene have been determined, using PCR amplification *Cfr*I enzyme. In order to pick up the *K232A* polymorphism, PCR - SSCP (Polymerase Chain Reaction - Single Strand Conformation Polymorphism) procedure has been applied according to Ripoli's [4] method. PCR amplification has been applied in 26 µl total volume. 12.5 µl 2X PCR Master Mix, 0.5 µM (forward primer 5'-GCACCATCCTCTTCCTCAAG-3' and reverse primer 5'-GGAGCGCTTTCGGATG-3'), 50 ng DNA sample has been used for each primer. In PCR procedure, the reproduction operation is done as such: 15 min on 95°C, 1 min on 94°C 1 min 35 rotations and 1 min on 60°C and 1 min on 72°C and the last elongation 3 min on 72°C with *Cfr*I enzyme and 411bp alanine variant division to 203 and 208 bp. The DNA bands are visualised in 2% agaroz gel painted with etidium bromide.

Microbiological Analyses

TAB (total aerobic bacteria), coliforms, *E. coli (Escherichia coli), L. monocytogenes (Listeria monocytogenes)* and *S. aureus (Staphylococcus aureus)* were determined for each milk sample. Microbiological analyses were performed according to FDA/BAM [8].

TAB: TAB was enumerated in PCA (Plate Count Agar) after incubation at 30°C for 48 h.

Coliforms: Coliforms were enumerated by surface plating on VRBA (violet red bile agar). Plates were incubated at 37°C for 24 h.

E. coli: E. coli were examined by surface plating on TBX (Tryptone Bile X - glucurunide) Agar. Colonies on plates incubated at 44°C for 24 h were enumerated.

S. aureus: S. *aureus* was determined by surface plating on BPA (Baird Parker Agar) supplemented with egg yolk-tellurite emulsion. Spread plates were incubated at 35°C for 46-48 h. Colonies with typical S. *aureus* morphology were examined microscopically following Gram staining and tested for catalase and coagulase activity.

L. monocytogenes: 25 g sample has been put in 225 ml BLEB (Buffered Listeria Enrichment Broth Base), incubated for 4 h in 30°C and after that selective agents and 25 mg/L natamisin has been added in the mediums and incubated for 48 h in 30°C. During the 24 h of the incubation period, Oxford and Palcam agars has been used and they have incubated for 48 h in 35°C. By the end of the 48th h of the incubation, *Listeria monocytogenes/ivanovii* has been passaged to Chromogenic Listeria Agar Base. Yeast Extract added trypticase soy agar (TSA) passages have been made from the colonies with the *Listeria* spp. suspicion and cultures have been purified. The suspicious isolates have been identified according to gram staining, catalase, motion, dextroglucose, malt sugar, rhamnose, mannitol, xylose fermentation, esculin hydrolization and nitrate reduction properties.

Quality Parameter Analyses

pH: Digital pH meter (Hannah Instruments) was been used to determine the pH values of all milk samples.

Milk fat rate: Gerber method was used to determine the milk fat. 10 ml concentrated H_2SO_4, 11 ml milk and 1 ml isoamyl alcohol have been put in Gerber tubes. Then, the mixture has been centrifuged in Gerber centrifuge at 2.500 rpm for 5 min and the milk fat amount has been directly read from Gerber tube scala [9].

Milk density: Lactodensimeter was been used in order to determine the concentration of the milk samples.

Milk acidity: Acidity rate of the samples is determined according to % lactic acidity. For this procedure, N/10 NaOH was used. 25 ml milk has been poured in a beher glass and 1-2 drops of phenolphthalein have been instilled on it and titration has been performed with the help of a burette. After the permanent pink colour was achieved, titration has been stopped, the spent aount of NaOH has been determined and calculation has been done. The calculation formula is:

A X 10 = T X 0.009

A: NaOH amount spent/ml, T: Acidity level in Thörner scale [10].

RESULTS

The PCR analyses have shown that 25 Holstein race cattles (experimental group) had *DGAT1* gene and the "K" and "A" alleles of the gene have high allele frequencies (between 0.93 and 0.95). On the other hand, the 24 native black cattles (control group) have been determined in order to have the *DGAT1* gene, but the gene has been identified to have low allele frequency (between 0.35 and 0.60). The control and experimental groups have been compared with regard to, milk microbiological charges according to the TAB, coliforms, *E. coli, L. monocytogenes, S. aurues*, and milk quality parameters (pH, fat rate, concentration and acidity). Milk samples have been collected weekly for a month. According to the findings, no milk sample has been determined to have *L. monocytogenes,* therefore this microbiologic parameter has not been included in the assesment. *Table 1* shows the testing of goup differences of the milk from control group and the experimental group according to the microbiological parameters while *Table 2* indicates the testing of group differences of the milk from two according to the chosen quality parameters. *Fig. 1* shows the DGAT1 gene from the Holstein cattles' milk, treated with ethidium bromide 2% agaroz gel DNA band views.

DISCUSSION

The milk productivity of the cattle is under the influence of multigenes and it is economically important to increase the milk productivity of livestock through correct genetic improvement. This study has been done to investigate the effect of the *DGAT1* gene in cattles on the microbiological quality of the milk and milk quality parameters.

DGAT1 gene lysine variant ("K" allele) is related to the reduction in protein and milk productivity. Increase in fat productivity is related to the alanine variant ("A" allele) is effective on the increase in milk and protein productivity and the reduction in fat productivity [2]. In the studies where Kaupe et al.[11], investigated *DGAT1* locus *K232* amplification on 1748 samples from 38 different cattle races, it has been determined that *DGAT1* "A" allele

Table 1. *The testing of goup differences of the milk from control group and experimental group according to microbiological parameters (The parameters written in bold carry statistically meaningful differences between the groups, P<0.005)*

Tablo 1. *Kontrol ve deney gruplarının sütlerinin seçilen mikrobiyolojik parametreler açısından grup farklılıklarının sınanması (Koyu karakterle yazılmış olan parametreler istatistik açıdan P<0.005 olduğundan gruplar arası anlamlılığı ifade etmektedir)*

Microbiological parameters / Statistical values	Mean	Standard Deviation	Standard Error Mean	Sig. (2-tailed)
Total Mesophilic Aerobic Becteria Count	-.0500	.16018	-.3743	-.0500
Coliforms	-.0448	.50134	.01602	.001
E. coli	-.0140	.33786	.01069	.000
S. aureus	19.771	-.0295	.01953	.030

Table 2. *The testing of goup differences of the milk from control group and the experimental group according to the chosen quality parameters (The parameters written in bold carry statistically meaningful differences between the groups, P<0.005)*

Tablo 2. *Kontrol ve deney gruplarının sütlerinin seçilen bazı süt kalite parametreleri açısından grup farklılıklarının sınanması (Koyu karakterle yazılmış olan parametreler istatistik açıdan P<0.005 olduğundan gruplar arası anlamlılığı ifade etmektedir)*

Milk quality parameters / Statistical values	Mean	Standard Deviation	Standard Error Mean	Sig. (2-tailed)
pH	6.62	.0168	.01388	.040
Milk Fat Rate	37.495	.2622	.04271	.000
Milk Concentration Rate	25.785	.0249	.00876	.014
Milk Acidity Rate	23.199	.0297	.01193	.019

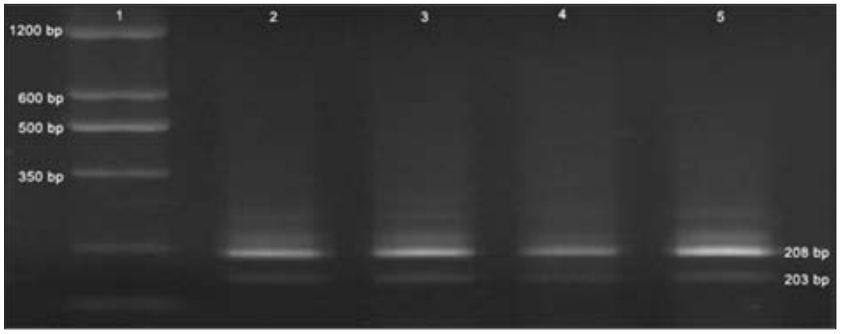

Fig 1. DGAT1 gene from the Holstein cattles, treated with ethidium bromide 2% agaroz gel DNA band views (partial sequence), (1: Marker, 2-5: The amplification products of the blood samples from the Holstein cattles)

Şekil 1. Holstein ırkı sığırlardan elde edilen DGAT1 geninin (parsiyel sekans olmak üzere) Ethidium bromide ile muamele edilmiş %2'lik agaroz jelde DNA bant görüntüleri, (1: Marker, 2-5: Holstein ırkı sığırların kan örneklerinden elde edilen amplifikasyon ürünleri)

frequency is high in meat types, whereas the related allele frequency is low in milk types. One of the few studies done in our country has shown that the *DGAT1* "K" allele and the *DGAT1* "A" allele frequencies are, respectively: Native Black 0.38-0.62 (N=73), Eastern Anatolian Red Cattle 0.25-0.75 (N=50), Western Anatolian Red Cattle 0.21-0.79 (N=48) and Grey Race 0.36-0.64 (N=49) and native races of Bos Indicus and Bostaurus centered in near east and African taurin-N'Dama cattle has higher *DGAT1* "K" allele frequencies [12]. Our findings are in line with Özdemir's reports and the *DGAT1* "K" and *DGAT1* "A" alleles gene frequencies of the native black cattles which have been reported as 0.35-0.60 (N=25). The obtained some milk quality parameters (milk fat rate, milk pH values, milk density and acidity) have shown that the "K" and "A" alleles of the *DGAT1* gene can directly affect the milk quality parameters. In addition to that, coliforms, *E. coli* and *S. aureus* is statistically and meaningfully less in the milk from the cattle with high allele frequency than in the milk from the cattle with low allele frequency.

Our findings show that *DGAT1* gene had a statistically meaningful effect on milk fat and milk concentration parameters. The study results indicate that the total fat in the milk of Holstein cattles which have a high allele frequency is higher than that of the milk of the native black race cattles which have a low allele frequency. According to this results, the native black race cattle's milk has higher density than the Holstein cattle's milk. Schennink et al.[3] have reported that the *DGAT1* gene is 50% effective in the quantity of the milk fat between the Holstein and Friesian cattle races. According to the results, milk fat in the milk from the cattle with high allele frequency is meaningfully higher than the milk from the cattle with the low allele frequency. Sun et al.[13] have pointed out that the "K" allele of the *DGAT1* gene increases the fat concentration of the milk but reduces the milk productivity. However, our findings in milk productivity parameter are not in parallel with Sun et al's findings. On the other hand, Mao et al.[1] have indicated that *DGAT1* gene is effective on fat concentration, total protein amount and total milk solid matter in cattles and have determined that "K" allele affects these parameters. The same researchers have also determined that the "A" allele has positive effects on milk productivity parameter. The findings of our study is similar to those of Mao et al.[1], about milk productivity. In our study, "K" and "A" alleles are determined in cattles with high allele frequency. According to our results, "A" allele is effective on milk productivity and it is thought to reduce/block the effect of "K" allele on milk productivity. The results show that the cattles with high allele frequency are "AK" haplotypes. This is relatively effective on the milk productivity of the cattles with low allele frequency but medical literature reports that "AA" haplotype cattles are more productive of milk than "AK" haplotype cattles whereas the milk of the cattles with the mentioned genomic profile includes less milk fat than the milk of the cattles of the "AK" haplotype [1].

In our study, the effect of the *DGAT1* gene on some of the milk microbiological parameters has also been investigated. There is no study in the literature showing whether the *DGAT1* gene has any effects on the microbiological qualities of milk or not. According to our findings, *DGAT1* gene is effective on coliforms and *E. coli* parameters and in the study, it has been determined that coliforms and *E. coli* is statistically meaningfully less in the milk of the cattles with the high allele frequency than that of cattle with the low allele frequency. Most of the coliforms and especially *E. coli* ferment milk sugar and sucrose during growthand produce acid and gas in result [14]. The milk of the cattles that had high allele frequency is thought to have relatively negative effect on the growth of coliforms and *E. coli*.

The increase of the proportion of total fat concentration in the solid matter of the milk and the dispersive distribution of the fat globules in the milk is probably another parameter which reduces the milk sugar and sucrose usage of the mentioned pathogens. Another probable reason that *E. coli* and coliforms grow relatively less in the milk of the cattles with high allele frequency might be that the optimum growth pH values of these pathogens are near to being neutral. According to the results, the pH values of the milk of the cattles with high allele frequency is meaningfully lower than those of the milk of the cattles with the low allele frequency. The average pH values of the milk of the cattles with high allele frequency (according to the general measures during the study period) is 6.3 whereas the average pH values of the milk of the cattles with low allele frequency (according to the general measures during the study period) is 6.7. The general pH value of

each group is accepted normal [15], however the difference between the group values is statistically meaningful. Although *E. coli* can grow on low acidic values as 4.4 pH when the other conditions are suitable, together with fat concentration parameter, low pH values are thought to be effective on *E. coli* and coliforms microorganisms. In the studied cattle groups, another microorganism determined to have statistically meaningful difference is *S. aureus*. Like coliforms and *E. coli, S. aureus* also ferments the milk sugars but differs from those microorganisms as it produces acid without gas during fermenting foremost mannitol and other sugars [14]. According to the results of the study, like the *E. coli* and coliforms, *S. aureus* growth is meanningfully less in the milk of the cattles with high allele frequency than that of the cattles with low allele frequency. The probable reasons which slow down the *E. coli* and coliforms growth in the milk of the cattles with the *DGAT1* gene are thought to be valid for *S. aureus* as well. The findings do not indicate a meaningful difference between the groups with regard to total mesofilic aerobic bacteria. No *L. monocytogenes* has been found in the milk samples examined, therefore this microbiological parameter is not included in the assesments.

Studies have identified genetic variation in the composition of milk quality parameters such as milk fat, density and acidity [16,17]. As a result, PCR methods are thought to be effective in determining the "K" and "A" alleles of the *DGAT1* gene. In addition to that, the findings of our study indicate that the quality parameters (pH, milk fat amount, milk concentration and acidity) of the milk of cattle with high allele frequency (Holstein race) have a statistically meaningful difference from the quality parameters of the milk of the cattles with low allele frequency. Also, it was determined that the examined milk microbiological parameters *E. coli, S. aureus* and coliforms grow meaningfully less in the milk of cattle with high allele frequency than in the milk of the cattles with low allele frequency. The *DGAT1* gene and especially the "K" allele of the gene is reported to be very ancient in European cattle races and to be inherited from the ancestors [2]. Medical literature reports that the native races also have the allele frequency but almost all these races have a lower allele frequency than the pureblood European races [18]. The correct strategies of hybridization and artificial insemination among the native races are thought to increase the allele frequency of the native races and thus contribute to the development of milk quality parameters and this is thought to positively effect the supply of more qualified milk to the consumer.

Our study is the first to examine the effect of allele frequencies of the *DGAT1* gene on the milk microbiological charges and no study similar to our study has been encountered in the literature. To supply high quality milk to the consumer is very important but the supply of raw milk with minimum microbiological risk is also a very important factor for safe milk supply to the consumer. According to the results of our study, the milk of the cattle races with high allele frequency is considered to be important in safer supply of milk to the consumer and the protection of public health. Similar studies on the subject and to define the biochemical mechanisms of the "K" and "A" alleles of the *DGAT1* gene more clearly is thought to be very rewarding with regard to the supply of both high quality and safe milk to the consumer.

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