POLYMORPHISM OF PROLACTIN (PRL), DIACYLGLYCEROL ACYLTRANSFERASE (DGAT-1) AND BOVINE SOLUTE CARRIER FAMILY 35 MEMBER 3 (SLC35A3) GENES IN NATIVE CATTLE BREEDS AND ITS IMPLICATION FOR TURKISH CATTLE BREEDING

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ABSTRACT

POLYMORPHISM OF PROLACTIN (PRL), DIACYLGLYCEROL ACYLTRANSFERASE (DGAT-1) AND BOVINE SOLUTE CARRIER FAMILY 35 MEMBER 3 (SLC35A3) GENES IN NATIVE CATTLE BREEDS AND ITS IMPLICATION FOR TURKISH CATTLE BREEDING

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In the present study samples from four native Turkish Cattle Breeds; South Anatolian Red (n= 48), East Anatolian Red (n= 34), Anatolian Black (n= 42) and Turkish Grey (n=46) and elite bulls of Holstein (n=21) were genotyped with respect to two milk production enhancer genes, Prolactin (*PRL*) and Diacylglycerol acyltransferase (*DGAT1*), and one disease (Complex Vertebral Malformation) causing gene (*SLC35A3*). A allele frequency for *PRL* gene, believed to be positively associated with the milk yield in cattle, ranged between 0.5645 (Anatolian Black) - 0.7558 (South Anatolian Red). K allele frequency which is thought to be related with the milk fat content in cattle varied between 0.7794 (East Anatolian Red) - 0.9250 (Anatolian Black). Complex Vertebral Malformation gene was not observed in any of the examined individuals (n= 164), hence, *SLC35A3* locus was monomorphic.

Pairwise Fst values based on the two polymorphic loci revealed that breeds are not significantly different from each other with respect to these two genes. Correlations, but weak, between the *PRL* A allele frequency and milk yield and similarly *DGAT1* K allele and milk fat content was observed, Principle Component Analysis generated two compound axis based on the two polymorphic loci. Positions of the breeds on the first axis were correlated with the milk fat content of the breeds, perfectly. Again, positions of the breeds on the second axis were correlated with the milk yield of the breeds. Furthermore, PCA revealed that both A of *PRL* and K of *DGAT1* genes seemed to have contributions in milk yield Results are believed to be useful for the management efforts of Turkish native cattle breeds.

Keywords: Native Turkish cattle breeds, *PRL*, *DGAT1*, *SLC35A3*, PCA.

ÖZ

PRL (PROLACTIN), DGAT1 (DIACYLGLYCEROL ACYLTRANSFERASE-1) VE SLC35A3 (BOVINE SOLUTE CARRIER FAMILY 35 MEMBER 3) GENLERİNİN POLİMORFİZİMİNİN YERLİ SIĞIR IRKLARINDA İNCELENMESİ VE TÜRK SIĞIR IRKLARININ ISLAHINDA UYGULAMALARI

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Sunulan çalışmada, dört yerli Türk sığır ırkı; Güney Anadolu Kırmızısı (n= 48), Doğu Anadolu Kırmızısı (n=34), Yerli Kara (42), Bozırk (n=46) ve damızlık olarak kullanılan Holstein (n=21) örnekleri, süt üretimini arttıran Prolaktin (*PRL*) ve Diaçilgliserol açiltransferaz 1 (*DGAT1*) genleri ve sığırlarda görülen genetik resesif bir hastalığa (Kompleks Vertebral Malformasyon) neden olan gen (*SLC35A3*) açısından çalışılmıştır. Yüksek süt verimiyle ilgili olduğu düşünülen *PRL* geninin A allelinin frekansının değeri 0.5645 (Yerli Kara) ile 0.7558 (Güney Anadolu Kırmızısı) arasında değişmektedir. Sütteki yağ miktarıyla ilgili olduğu düşünelen *DGAT1* geninin K allelinin frekansının değeri 0.7794 (Doğu Anadolu Kırmızıs) ile 0.9250 (Yerli Kara) arasında değişmektedir. Kompleks Vertebral Malformasyon geni, çalışılan hiç bir örnekte (n= 164) tespit edilmemiştir. Sonuç olarak *SLC35A3* geninin monomorfik olduğu gözlenmiştir.

İki polimorfik lokusa dayalı ikili Fst değerleri, ırkların bu iki gen açısından biribirinden anlamlı bir şekilde farklı olmadığını göstermektedir. *PRL* A alleli frekansı ve süt üretimi arasında ve *DGAT1* K alleli frekansı ve süt yağı miktarı arasında zayıf olmakla birlikte bir bağlantı gözlenmiştir. Temel öğeler analizi (PCA) sonucunda iki polimorfik lokusa dayalı iki bileşik eksen oluşmuştur. Birinci eksendeki ırkların konumu, ırkların sütteki yağ miktarıyla tam bir korelasyon göstermiştir. Aynı şekilde ikinci eksendeki ırkların konumu, ırkların süt üretim miktarıyla gene tam korelasyon göstermiştir. Ayrica, temel öğeler analizi süt miktarina *PRL* A alleli gibi *DGAT1* K allelinin de etkisi olabileceğini göstermiştir. Sunulan çalışmanın sonuçlarının, yerli Türk sığır ırklarının iyileştirilmesine katkıda bulunacağı düşünülmektedir.

Anahtar Kelimeler: Yerli Türk Sığır Irkları, *PRL*, *DGAT1*, *SLC35A3*, PCA

To my brother Ali Kemal Kepenek…

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B: Beta

κ: K

°C: Degrees Celsius

µl: microliter

µM: Micromolar

A: Adenine

bp: base pair

C: Citosine

ddATP: Dideoxyadenosine Triphosphate

ddCTP: Dideoxycytidine Triphosphate

ddGTP: Dideoxyguanosine Triphosphate

ddNTP: Dideoxynucleotide Triphosphate

ddTTP: Dideoxythymidine Triphosphate

DGAT1: Diacylglycerol acyltransferase 1

dH2O: Distilled water

DNA: Deoxyribonucleic acid

DnaSP: DNA Sequence Polymorphism

dNTP: Deoxyribonucleotide Triphosphate

EDTA: Ethylene diamine tetra acetic acid

EtOH: Ethanol

e.g: For example

G: Guanine

K3EDTA: Potassium EDTA

MgCl2: Magnesium Chloride

mg: miligram

ml: Mililiter

mM: Milimolar

M: Molar

NaAc: Sodium Acetate

ng: Nanogram

pca: Principle Component Analysis

PCR: Polymerase Chain Reaction

PRL: Prolactin gene

pp: Page

pmol: picomoles

Rpm: Rotations per minute

S: Segregating Sites

SDS: Sodium dodecyl sulfate

SLC35A3: bovine solute carrier family 35 member 3 gene

T: Tymine

TAE Buffer: Tris Acetate EDTA Buffer

UV: Ultra Violet

V: Volt

CHAPTER 1

INTRODUCTION

Cattle belong to the Bovidae family which appeared in the Miocene approximately 20 MYA, during the evolution of mammals. Ancestral species of domesticated cattle was Bos *primigenius*. Bos *primigenius* believed to harbor two taxa, *Bos taurus* and *Bos indicus* (Bradley *et al.*, 2005). It is known that for the individuals of the species of pre-domesticated ones are bigger in size compared to domestics. Hence, size reduction is considered as one of the signs of domestication. From the archeological finds, it was observed that first size reduction in *Bos taurus* (European taurine cattle) was observed in the middle west banks of Euphrates River (Peters *et al.,* 1999 referred by Bradley *et al.,* 2005) within the Mid Pre-Pottery Neolithic B (ca. 8500 BP uncalibrated). Domestics at the center of domestication must be harboring the highest level of genetic diversity. It can be anticipated that, diversity was reduced due to migration in the direction away from the domestication center by the founders (Nichols *et al.*, 2001).

As in this line, it is known that European domestic cattle, almost exclusively, went from Anatolia (Troy *et al.,* 2001) and they have low level of diversity (Loftus *et al.,* 1999) Because of the geographic proximity of the Turkish native breeds to the region of putative domestication area of European cattle, they might still be harboring high level of genetic diversity as supported by a recent study (Freeman *et*

al., 2005) and presumably at least part of this diversity is the "raw" diversity and has the potential to serve for the humans in the future especially under new emerging environments. Therefore, Turkish native breeds must have high priority in conservation (Bruford *et al.,* 2003).

However, recently, Turkish native cattle breeds have been widely inseminated with semen of elite bulls, predominantly by Holstein bulls. Primary purpose of this introgression is to increase the milk yield in cattle in Turkey. During this introgression process gene pools of Turkish native breeds must be changing. Fertilization of native cows with relatively few males must be reducing the effective population size of the gene pools and therefore genetic diversity might be in loss with a high rate. Furthermore, some alleles which could be used in the future might be in the process of loss. To understand the effect of introgression, composition of genetic diversity for many loci must be determined and monitored. In the present study, the state of diversity in four native Turkish cattle breeds; Turkish Anatolian Black, South Anatolian Red, East Anatolian Red and Turkish Grey and in improved breeds of Holstein breed with respect to *PRL* and *DGAT1* and *SLC35A3* genes were tried to be determined. Further, follow up studies will document the rate of change in the gene pools with respect to the genes under consideration.

1.1 Prolactin *(PRL),* Diacylglcerol acyltransferase *(DGAT1)* and Complex Vertebral Malformation (CVM) syndrome *(SLC35A3)* genes

1.1.1 Prolactin (*PRL*) gene

Prolactin, pertaining the Prolactin/ Growth Hormone/ Placental Lactogen Family, is a polypeptide hormone which is synthesized in and secreted from specialized cells of the anterior pituitary gland, the lactotrophs (referred by Freeman *et al.,* 2007). It

was found in many mammals including rats, primates, rodents, sheep, pig, mice and human. Also it was synthesized by many organs other than anterior pitutary gland: Brain, placenta, amnion, decidua, uterus and mammary gland. It had major role in lactogenesis, lactation, mammary growth (Kopecny *et al.,* 1998 referred by Skinkyte *et al.,* 2005).

Bovine Prolactin gene *(PRL)* is located on chromosome 23 and composed of five exons and four introns (Camper *et al.*, 1984 referred by Skinkyte *et al.,* 2005). A silent A-G mutation in codon for amino acid 103 in exon 3 of bovine *PRL* gene led to a polymorphic *RsaI* site (Lewin, 1992 referred by Skinkyte *et al.*, 2005). Lewin *et al.* (1992) first presented the polymorphism laeding *RsaI* enzyme restriction site of Bovine *PRL* gene which affects the milk production traits including milk yield, milk fat yield and milk protein yield. Later, significant effect of *PRL* locus on milk production traits was exhibited by Chung *et al.* (1996, referred by Dybus, 2002).

Distribution of *PRL- RsaI* allele, A allele of Prolactin gene *(PRL)* in cattle breeds were studied by many researchers. A allele frequency for many breeds were given below. Udina *et al.,* (2001) showed that A allele frequencies of *PRL* gene were 91.4, 85.9 and 80.0 in Gorbatov Red breed, Ayrishire breed and Black Pied breed, respectively. Another study on this subject was done by Dybus (2002). A allele frequencies of *PRL* gene were 0.85, 0.85, 0.86, 0.83 and 0.88 in Kosierzewo, Nowielice, Smardzewo, Trzebusz and Ostrowiec breeds, respectively. In the study done by Skinkyte *et al.,* (2005), A allele frequencies were 0.79 and 0.87 in Lithuanian Black and White and Lithuanian Red breeds, respectively. Polymorphism of *PRL* gene in Argentina and Bolivian Creole cattle was studied by Liron *et al.,* (2002). Results indicated that A allele frequencies of *PRL* gene were 0.96, 0.95, 0.81, 0.98, 1.00 and 0.90 in Argentine Creole, Patagonian Creole, Saavedreno, Chaqueno, Boliviano, Yacumeno and Chusco breeds respectively. Similarly, in Khatami *et al.'s* (2005) study A allele frequencies of *PRL* gene were found 0.95, 0.61 and 0.65 in Russian Black and White, German Black and White and Yaroslavl breeds, respectively. Again, distribution of polymorphism of *PRL*

gene was studied by Miceikiene *et al.,* (2006), A allele frequencies of *PRL* gene were 0.97, 0.95, 0.77 and 0.85 in Lithuanian White Backed, Lithuanian Light Grey, Lithuanian Red and Lithuanian Black and White breed, respectively in this study.

Finally, in two independent studies A allele frequency of *PRL* gene was high, 0.95 in Holstein breed which is a well known breed selected for high milk yield (Chung *et al.,* 1996 and Chrenek *et al.,* 1998 referred by Miceikiene *et al.,* 2006).

As it can be seen from the summarized data, based on the breeds examined, A allele frequency is varying between 0.61 (German Black and White), 0.97 (Lithuanian White Backed) in Europe and is more often towards the upper end of the range. And there was no previous observation of *PRL* gene frequency in Turkish native breeds.

1.1.2 Diacylglycerol acyltransferase -1 *(DGAT1)* gene

Diacylglycerol acyltransferase1 *(DGAT1)* gene encodes an enzyme which plays a major role in the synthesis of triglycerides. Triglycerides which are major components of fat are formed by binding of diacylglycerol to long chain fatty acyl-CoAs. This reaction is catalyzed by at least two enzymes. One of these enzymes is encoded by *DGAT1* (homolog1, mouse) (Cases *et al.* 2001 referred by Winter *et al.,* 2002). Lactation deficiency was observed in female mice lacking both copies of *DGAT1,* probably as a result of impaired or deficient triglyceride synthesis in mammary gland. After this observation *DGAT1* gene was suggested as a functional candidate gene for milk production traits (Smith *et al.,* 2000 referred by Winter *et al.,* 2002). *DGAT1* gene was localized on centromeric end of the bovine chromosome 14. A base substitution *(K232A)* in the *DGAT1* (the acyl-CoA: diacylglycerol acyltransferase1) gene at position 10,433 and 10,434 in exon number 8 lead to QTL (quantitative trait loci) variation (Grisart *et al.,* 2002). While Lysine variant (K allele) of *DGAT1* gene was associated with high fat yield, Alanine variant (A allele) of *DGAT1* gene was associated with high milk yield.

DGAT1 polymorphism was first detected by Spelman *et al.*, (2002). In one of the study Winter *et al.*, (2002) it was observed that frequency of Lysine variant (K) was ranging between 0.10 to 1.00 in the 16 breeds examined. Lowest K allele (Lysine) frequency (0.1) was found in Fleckvieh breed (*Bos taurus taurus*) while highest K allele frequency (1.0) was found in Water buffalo (*Bubalus bubalus*).

Another extensive study on this subject was performed by Kaupe *et al.,* (2003). In the study *DGAT1* polymorphism in *Bos indicus* and *Bos taurus* cattle breeds were analyzed using 1748 DNA samples of 38 different *Bos taurus* covering four native Turkish cattle breeds and *Bos indicus* cattle breeds from 13 countries of five continents. Kaupe *et al.,* (2003) observed that beef breeds tended to exhibit higher *DGAT1* A allele frequencies, yet, in dairy cattle the range for the A allele was from very low levels to unexpectedly high level. Turkish Anatolian Black, South Anatolian Red, East Anatolian Red and Grey Step exhibited intermediate K allele frequencies (21-38%). In another study, Polymorphism of *DGAT1* variants and milk production traits in German cattle breeds was studied by Thaller *et al.,* (2003). Frequencies of lysine variants were 0.072 and 0.548 in Fleckvieh and German Holstein breeds, respectively. Lower lysine frequency of Fleckvieh breed was an unexpected observation. Because, in the past Fleckvieh breed was the most preferred for its milk content (including high fat) while German Holstein which contains higher lysine frequency had higher milk yield. Presumably, observed results are due to the recent selections towards the high milk yield for Fleckvieh and high content for Holstein.

Another unexpected result was observed by Sanders *et al.,* (2006). K allele frequency of *DGAT1* gene was 0.61 in German Angeln breed which was relatively high for a European breed. Again it was proposed that high K allele frequency could be the result of recent selection for the milk content traits in this breed.

Furthermore Lacorte *et al.,* (2006) studied *DGAT1* polymorphism in Brazilian breeds. K allele frequencies of *DGAT1* gene ranged from 0.96 to 1 in four different Zebu breeds. It was 0.27 in Holstein breed while it was 0.27 in Gyr x Holstein F_1.

In Jersey breed K allele frequencies of *DGAT1* gene ranged 0.69 to 0.88 (Spelman *et al.,* 2002, Winter *et al.,* 2002 and Kaupe *et al.,* 2003).

Furthermore, in the Holstein breed, allele frequencies of lysine variant ranged from 0.3 in the New Zeland to 0.63 in the Ducth population (Bovenhars and Schrooten 2002, Grisart *et. al.,* 2002). Lysine variant of *DGAT1* were estimated 0.35 (Winter *et al.,* 2002) and 0.44 (unpublished data Thaller *et al.*) in German Holstein. The difference could be associated with different genetic backgrounds or different selection directions.

As a summary a large proportion of breeds exhibited low to intermediate allele frequencies for K allele. Lower K allele frequency was observed in *Bos taurus* cattle than that of *Bos indicus* cattle. While fixation of A allele in *Bos taurus* breeds was observed, fixation of K allele was observed in one *Bos indicus* breed as well as in another species Water buffalo *(Bubalus bubalus*).

1.1.3 Complex Vertebral Malformation (CVM) syndrome

Complex Vertebral Malformation (CVM) is a hereditary lethal disease observed in Holstein calves. The syndrome presents many anomalies (Nagahata *et al.*, 2002) including shortened cervical and thoracic regions of vertebral column, bilateral symmetric contraction of the metacarpophalangeal and metatarsophalangeal joints, and symmetric arthrogryposis.

The syndrome was first discovered in the Danish Holstein population by Agerholm *et al.*, (2001). In their study, Holstein calves were analyzed by using morphological variations, radiological findings and pedigree analyses. Agerholm *et al.,* (2001)

pointed out that all CVM cases took place in a familial pattern with extensive inbreeding because affected calves were progeny of apparently normal parents. It was suspected that there was a homozygous locus having alleles originated from common ancestor. The bull Carlin-M Ivanhoe Bell was used worldwide because of superior lactation performance. It is believed that he and his daughters are responsible in distributing the syndrome worldwide. Shortly after identification of CVM in Denmark Holstein population, many cases were reported about existence of CVM in the United States, the United Kingdom, and in Japan (Duncan *et al.,* 2001, Revell, 2001, Nagahata *e. al*., 2002 referred by Thomsen *et al.,* 2005). Genetic identification of the gene which is responsible for the CVM disease was done by Bendixen *et al.,* (2002). Now, it is known that the syndrome is autosomal recessively inherited defect and a G to A point mutation at nucleotide position 559 of the *bovine solute carrier family 35 member 3 (SLC35A3)* led to this disease (Thomsen *et al.,* 2005).

When Nielsen *et al.,* (2002) and Malher *et al.,* (2006) analyzed the effect of fertility traits in CVM carrier cows they observed that risk of return to service increases and frequency of calving after first insemination were reduced, in Holstein cattle. Furthermore, 93% of CVM affected calves died before the end of the gestation period and many of the fetuses were aborted after 100-120 days conception. At this phase of lactation a cow did not maintain a high yield until the following calving. Furthermore, Agerholm *et al.*, (2004) showed that incidence of CVM affected calves is less than that of expected, possibly due to the high intrauterine mortality. Because of all these reasons, culling ratio of cows was highly increased in these carrier cows. population size of the gene pools and therefore genetic diversity might be in loss with a high rate. Furthermore, some alleles which could be used in the future might be in the process of loss. To understand the effect of introgression, composition of genetic diversity for many loci must be determined and monitored. In the present study, the state of diversity in four native Turkish cattle breeds; Turkish Anatolian Black, South Anatolian Red, East Anatolian Red and Turkish Grey and in elite bulls of Holstein breed with respect to *PRL* and *DGAT1* and *SLC35A3* genes were tried

to be determined. Further, follow up studies will document the rate of change in the gene pools with respect to the genes under consideration.

1.2 The Objective of the Study

Nowadays, molecular genetic markers are widely used for characterization of milk production traits in dairy cattle, and also they are used for detection of genetically inherited diseases.

The milk production Prolactin *(PRL)* and Diacylglycerol acyltransferase *(DGAT1)* genes are the two major genetic markers used for the characterization of milk production traits. On the other hand, Complex Vertebral Malformation (CVM) is the autosomal, recessively inherited lethal disease due to a point mutation in *SLC35A3* gene. Hence, the form of the products of these 3 genetic markers (*PRL, DGAT1* and *SLC35A3*) is economically important in cattle and must be examined in native Turkish cattle breeds. If for example A allele frequency of *PRL* is low, to have a higher milk yield it might be subjected to a directional selection to increase its frequency in Turkish cattle. On the other hand introgression of Holstein into Turkish breeds might be introducing CVM related allele which might be causing economical harms through the carrier cows.

In the present study genetic characterization of four native Turkish breeds (Grey Step, East Anatolian Red, Native Black and South Anatolian Red) and elite bulls of Holstein breed which is sampled from Cattle Breeders Association of Turkey were done with respect to *PRL*, *DGAT1* and *SLC35A3* genes. Results were employed to comparatively evaluate the milk yield and fat content properties of native breeds and those of other breeds which are available in the literature with respect to the two loci: *PRL* and *DGAT1*. Also, potential threat for the contamination of native gene pools by the genes from Holstein breed with respect to CVM related allele of *SLC35A3* gene was discussed. Produced data is believed to contribute in

conservation and management of Turkish native cattle breeds, which are the reservoirs of genetic diversity known to have prime importance for the future of humankind.

CHAPTER 2

MATERIALS AND METHODS

2.1 Samples

In the present study, four native Turkish cattle breeds were examined. Their distribution is as follows: South Anatolian Red (SAR) is found in South of Taurus Mountains and South Anatolia. Turkish Grey (TG) is distributed in Thrace and other cities of North-West Anatolia; East Anatolian Red (EAR) is distributed in East and North East Anatolia, whereas Anatolian Black (AB) can be found in Middle and North Anatolia. Altogether, 170 individuals from native breeds were examined. Samples of SAR breed were obtained from Çukurova Agricultural Research Institute. Fourty two samples of AB breed were collected from the villages around Çankırı and Kastamonu. Thirty four samples of EAR breed were obtained from Institute of East Anatolian Agricultural Research. Fourty six samples of TG breed were obtained from Marmara Agricultural Institute and from villages around the Keşan. Twenty one samples of Holstein breeds were obtained from Lüleburgaz Türkgeldi Agricultural Administration. The number of collected samples, number of samples genotyped for each locus (*PRL*, *DGAT1*, and *SLC35A3*) was given in Table 2.1. All samples were collected by the two staff members of Namik Kemal University; Prof. Dr. İhsan Soysal and Dr. Emel Özkan in 2002. Blood samples were stored at +4 °C during the transportation and then stored at +4 °C until they

were used for DNA extraction. DNAs of 191 (170 native 21 Holstein) individuals were genotyped for two milk performance enhancer genes, (*PRL* (n=176) and *DGAT1* (n= 122)) and one disease related gene (*SLC35A3* (n=164)).

Table 2.1 The number of collected samples, number of samples genotyped for each locus (PRL, DGAT1, and SLC35A3) and number of samples genotyped for at least two loci.

Breed	Number of collected samples	Number of genotyped samples			Number of samples genotyped for at least two loci
		PRL	*DGAT1*	*SLC35A3*	
Turkish Grey	46	45	30	40	28
East Anatolian Red	34	34	34	34	34
Anatolian Black	42	31	20	32	17
South Anatolian Red	48	45	38	37	31
Holstein	21	21	0	21	21

2.2 Laboratory Experiments

2.2.1 DNA Extraction

DNA isolation from whole blood sample was done by employing phenol-chloroform-isoamylalcohol (25:24:1) extraction method. DNAs were extracted from 10 ml blood by using Sambrook *et al.*'s (1989) method.

Blood samples were collected in the tubes containing EDTA to prevent coagulation and completed up to 50 ml with 2X lysis buffer to lyses the red blood cells. Tubes

were mixed for 10 minutes by inversion and then centrifuged at 3000 rpm for 10 minutes at +4 °C to precipitate nuclei. The pellet was resuspended in 3 ml of salt-EDTA buffer by vortex. Then 0.3 ml of 10% SDS and 150 µl proteinase-K(10mg/ml) were added and the tubes were incubated at 55 °C for 3 hours. After the incubation, 3ml phenol was added and this mixture was centrifuged at 3000 rpm for 10 minutes at +4 °C. The supernatant was transferred to new falcon tubes mixed with 3 ml phenol–chloroform–isoamylalcohol solution (25:24:1) and centrifuged at 3000 rpm for 10 minutes at +4 °C. Then the upper phase was collected carefully and transferred into a new glass tube by a transfer pipette. About 2 volumes of ethanol were added to precipitate the DNA. Then mixture was mixed gently by inversion. After that, precipitate was hooked out. Finally, precipitated DNA was dissolved in 0.5 ml of 10 mM Tris and 1mM EDTA solution (pH: 7.5). After isolation, DNA was stored at -20 ºC.

2.2.2 Checking the Presence of DNA

After DNA extraction, existence and concentration of DNA was checked by agarose gel electrophoresis. 0.8% agarose gel was prepared by boiling agarose in 0.5X Tris-Boric Acid-EDTA (TBE) buffer. The prepared gel was poured into an electrophoresis plate and left at room temperature for about 30 minutes for polymerization. 1µl of newly isolated genomic DNA, 6 µl of 6X loading buffer (bromophenol blue dye) and 6 µl of dH_2O were mixed and then loaded into the wells of the gel. The DNAs were run on agarose gel at 100 V for about 30 minutes in 0,5X TBE buffer, stained in 0.5 µl/ml ethidium bromide (Et-Br) solutions and then was visualized under UV light. The presence or absence of smears and migration patterns of the bands on the gel corresponds to presence and the quality of DNA.

2.2.3 Amplification of *PRL*, *DGAT1* and *SLC35A3* Genes by Using Polymerase Chain Reaction (PCR)

The polymerase chain reaction (PCR) is a technique widely used in molecular biology. The procedure consists of 3 main steps: denaturation, annealing and extension. In the denaturation step, double stranded DNA is dissociated into single strands and then in the annealing step, primers are annealed to single stranded DNAs. After that, in the extension step, oligonucleotide primers are extended in the 5'- to- 3' direction by *Taq* polymerase using the single stranded DNA bound to primer as a template. After these reactions, the enzymatic amplification of microgram quantities of specific DNA regions was obtained (Klug and Cummings, 1997).

In the present study, three different gene regions containing *PRL*, *DGAT1* and *SLC35A3* (CVM disease related gene) genes were amplified with PCR.

2.2.3.1 *PRL* gene

A 156-bp fragment of *PRL* gene was amplified using PCR procedure applied by Dybus (2002) with 50 µl reaction mixture containing;

- PCR buffer 1.0 X
- $MgCl_2$ 3.0 mM
- dNTP 0.2 µM
- Primer (both forward and reverse primers) 0.1 Pm
- DNA 50 ng
- Taq polymerase 0.5 U

Primers given below were used for amplification of a 156-bp fragment of *PRL* gene.
Forward: 5' - CGAG TCCTTATGAGCTTGATTCTT- 3'
Reverse: 5' – GCCTTCCAGAAGTCGTTTGTTTTC - 3'
PCR conditions used for the amplification of the *PRL* gene were given in Table 2.2.

Table 2.2 PCR condition of the PRL gene.

Step	Temperature	Duration	Number of Cycles
Denaturation	94 °C	3 minutes	1
Denaturation	94 °C	20 seconds	35
Primer annealing	53 °C	40 seconds	
Synthesis	72 °C	40 seconds	
Final Extension	72 °C	10 minutes	1

2.2.3.2 *DGAT1* gene

A 411-bp fragment of bovine *DGAT1* gene was amplified using PCR procedure applied by Spelman *et al.* (2000) with 20 µl reaction mixture containing;

- PCR buffer 1.0 X
- $MgCl_2$ 0.6 mM
- dNTP 0.2 µM
- Primer (both forward and reverse primers) 1.0 pmol
- DNA 50 ng
- Taq polymerase 1 U

Primers given below were used for amplification of 411-bp fragment of the bovine *DGAT1* gene;

Forward: 5'- GCACCATCCTCTTCCTCAAG-3'

Reverse: 5'- GGAAGCGCTTTCGGATG -3'

PCR condition used for the amplification of the *DGAT1* gene was presented in Table 2.3.

Table 2.3 PCR condition of the DGAT1 gene.

Step	**Temperature**	**Duration**	**Number of Cycles**
Denaturation	95 °C	5 minutes	1
Denaturation	94 °C	60 seconds	35
Primer annealing	60 °C	60 seconds	
Extension	72 °C	60 seconds	
Final Extension	72 °C	10 minutes	1

2.2.3.3 *SLC35A3* gene

A 233-bp of *SLC35A3* gene was amplified using PCR protocol applied by Kanae *et al.* (2005) with 30 µl reaction mixture containing;

- PCR buffer 1 X
- $MgCl_2$ 1.5 mM
- dNTP 0.2 µM
- Primer (both forward and reverse primers) 0.3 pm
- DNA 50 ng
- Taq polymerase 0.5 U

Primers given below were used for amplification of 233-bp of bovine *SLC35A3* (CVM disease related gene) gene;
Forward: 5'- CACAATTTGTAGGTCTCACTGCA-3'
Reverse: 5'-CGATGAAAAAGGAACCAAAAGGG-3'

PCR conditions used for the amplification of the *DGAT1* gene were given in Table 2.4.

Table 2.4 PCR condition of the DGAT1 gene.

Step	Temperature	Duration	Number of Cycles
Denaturation	94 °C	3 minutes	1
Denaturation	94 °C	45 seconds	30
Primer annealing	55 °C	20 seconds	
Extension	72 °C	40 seconds	
Final Extension	72 °C	10 minutes	1

2.2.4 Checking the Presence of PCR Products

The presence of PCR products were analyzed by using agarose gel electrophoresis. 2% agarose gel was prepared by boiling agarose in 0,5X TBE buffer, pouring it into an electrophoresis plate and leaving it at RT (room temperature) for 30 minutes for polymerization. Then, 5µl of 6X bromophenol blue dye was added to 5µl of each PCR product and loaded into the wells of the gel. The PCR products in the wells were run in 0,5X TBE buffer at 120V until the bands reached the end of the gel. Then, the gel was stained in 0.5 µl/ml Ethidium Bromide (Et-Br) solution and the PCR products were directly visualized by UV fluorescence. The photograph of the gel was obtained by a gel image system.

2.2.5 Enzyme Digestion and Detection of Genotypes

2.2.5.1 *PRL* gene

A 156-bp of *PRL* gene was amplified by using the PCR procedure mentioned above. Amplified *PRL* gene PCR products were digested using procedure applied by Dybus (2002). After PCR amplification, amplified 156-bp fragment of *PRL* gene was overnight digested with *RsaI* enzyme, then were run on 6% polyacrylamide gel and visualized under UV with the help of ethidium bromide. Genotyping of each individual for *PRL* gene was was done as shown in Figure 2.1.

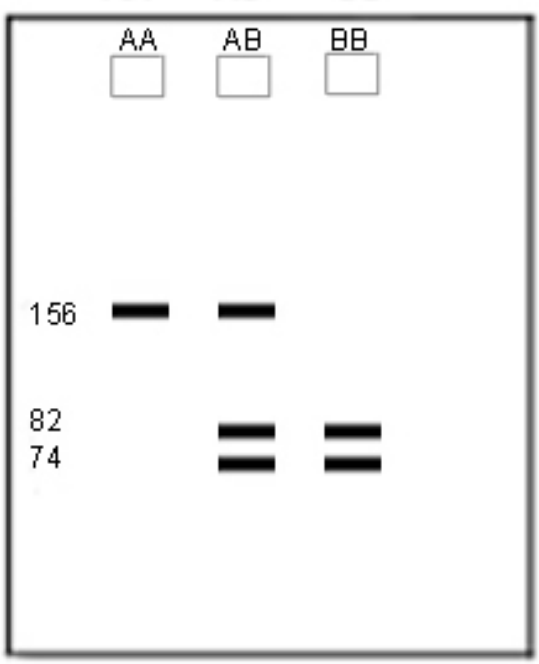

Figure 2.1 The expected genotypes and the sizes of the expected restriction fragments for the *PRL* gene on 6% polyacrylamide gel.

PRL gene has two alleles denoted as A and B. The B allele of the *PRL* gene has a restriction site at codon for amino acid 103 for *RsaI* restriction enzyme. Therefore, as a result of digestion of 156-bp fragment of *PRL* gene with *RsaI* restriction enzyme, three different genotypes were expected (Lewin *et al.*, 1992 referred by Dybus 2002). Figure 2.1 visualizes the expected genotypes when *PRL* gene was digested with *RsaI* restriction enzyme. If both of the alleles of the *PRL* genes were not restricted with *RsaI* enzyme, just 156-bp band was expected. These individuals were genotyped as AA. If just one of the gene copies had a restriction site then the156-bp, 82-bp and 74-bp fragments were expected and individuals having this74-bp and 82-bp long bands were genotyped as BB genotype.

2.2.5.2 *DGAT1* gene

In the presented study, 411-bp fragment of *DGAT1* gene was amplified and digested with *CfrI* restriction enzyme. Digestion products were separated electrophoretically on 4% agarose gel.

A point mutation from lysine to alanine (K232A) results in two alleles of the *DGAT1* gene (K and A). The allele that has lysine at position 10433 or 10434 was denoted as the K allele. The mutation from lysine to alanine cause the formation of a restriction site for *CfrI* restriction enzyme and this allele was denoted as A alele.

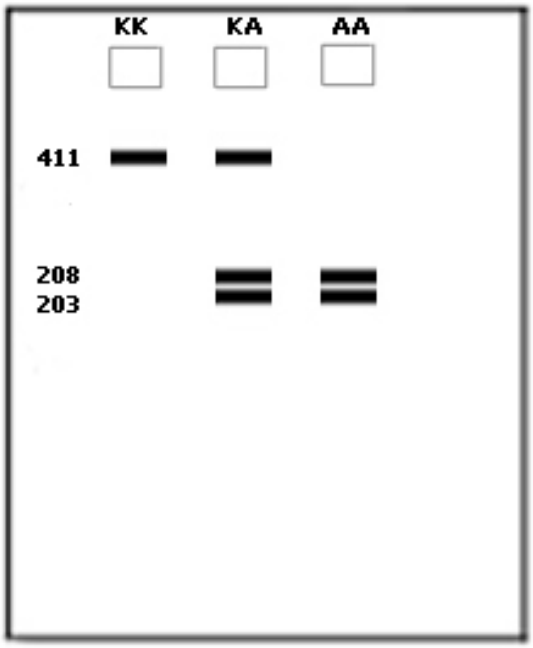

Figure 2.2 The expected genotypes and the sizes of the expected restriction fragments for the DGAT1 gene on 4% agarose gel.

Three different genotypes were expected with the *CfrI* digestion of *DGAT1* gene (Figure 2.2). As a result of the digestion, if both of the alleles of the *DGAT1* genes were not rectricted with C*frI* restriction enzyme, only 411-bp long band was expected. These individuals were genotyped as KK. If just one of the gene copies had restriction site then three fragments (411-bp, 208-bp and 203-bp) were expected. These individuals were genotyped as the ones that have KA genotype. In the case of the presence of restriction sites on both copies of *DGAT1* gene, it was expected to observe only 208-bp and 203-bp bands and individuals exhibiting these two bands were genotyped as AA genotype. However, since there is only 5 bp difference between 208-bp and 203-bp, these two DNA fragments (208-bp and 203-bp) were visualized as one band on 4% agarose gel (see Figure 3.5).

2.2.5.3 SLC35A3 gene

Amplified 233-bp of *SLC35A3* gene was digested with *EcoT22* enzyme using procedure applied by Kanae *et al.* (2005). Digestion products were separated electrophoretically in 4% agarose gel and then visualized under UV light using ethidium bromide dye.

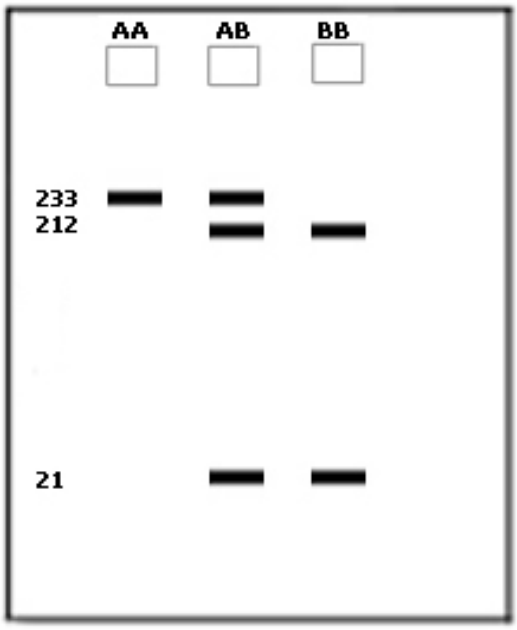

Figure 2.3 The expected genotypes and the sizes of the expected restriction fragments for the SLC35A gene on 4% agarose gel.

As shown in Figure 2.3 with the digestion of 233-bp fragment of *SLC35A3* gene, three different genotypes were expected (Bennewitz *et al.*, 2001). From the two alleles (A and B) of the *SLC35A3* gene, B has a restriction site for *EcoT22*

restriction enzyme. If both of the alleles of the *DGAT1* genes did not contain *EcoT22* restriction site, only 233-bp long band was expected. These individuals were called as AA genotype and individuals having AA genotype were considered as healthy individuals (Bennewitz *et al.,* 2001.). If just one of the gene copies was restricted then the 233-bp, 212-bp and 21-bp fragments were expected and genotyped as AB. Individuals having AB genotype were considered as carriers (Bennewitz *et al.,* 2001.). In the case of the presence of restriction sites on both copies of *SLC35A3* gene, 212-bp and 21-bp bands were expected. Individuals exhibiting these two bands were genotyped as BB genotype and considered as the ones that have CVM disease (Bennewitz *et al.,* 2001*).*

2.3 Statistical Analyses

2.3.1 Allele frequencies and heterozygosities

Allele frequency is a measure of the relative frequency of an allele of a genetic locus in a population and presents the genetic diversity of a species population. In the present study, there were 2 alleles for *PRL* (A and B), *DGAT1* (K and A) and *SLC35A3* (A and B) genes. Allele frequencies of each allele were calculated in the studied five populations (Turkish Grey, East Anatolian Red, Anatolian Black, South Anatolian Red and Holstein populations). Allele frequencies were calculated according to the formula given below;

$$\text{Frequency of an allele (p)} = \frac{(2 \times \text{number of homozygote}) + (\text{number of heterozygote})}{2 \times \text{total number of individuals (N)}}$$

pattern were genotyped as the AB. In the case of the restriction of both of the copies of *PRL* gene 74-bp and 82-bp long bands were expected. Individuals exhibiting Heterozygosity which presents the measurement of genetic variation in a population was calculated with the formula given below;

$$\text{Observed Heterozygosity} = \frac{\text{Number of heterozygotes}}{\text{Number of heterozygotes (N)}}$$

Frequencies of alleles and the observed heterozygosities for *PRL*, *DGAT1* and *SLC35A3* genes for Turkish Grey, Anatolian Black, East Anatolian Red, South Anatolian Red and Holstein breeds were calculated by using Arlequin (Excoffier *et al.*, 2005) package program.

Presence of Hardy-Weinberg (HW) equilibrium in Turkish Grey, Anatolian Black, East Anatolian Red and South Anatolian Red breeds for *PRL*, *DGAT1* and *SLC35A3* genes were checked by calculating the expected genotype frequencies and comparing them with the observed ones. HW equilibrium is based on the following assumptions: (i) mating is random, (ii) allelic frequencies are conserved from generation to generation, (iii) no significant migrations occur, (iv) mutation, selection, genetic drift and gene flow are negligible (Hedrick, 2000). All of these requirements were assumed to be true while calculating the expected genotype frequencies, which were calculated as follows;

Expected frequency of homozygote = p^2 and q^2
Expected frequency of heterozygotes (Expected heterozygosity) = $2pq$,
Where; $p + q = 1$

The significance of the departure from Hardy-Weinberg equilibrium was tested with Arlequin package program (Excoffier *et al.*, 2005) based on the Guo and Thomson (1992) procedure. The test was analogous to Fisher's exact test on a two-by-two

contingency table, but extended to a triangular contingency table of arbitrary size. It is done using a modified version of the Markov-chain random walk algorithm described Guo and Thomson (1992).

2.3.2 The Fst analysis

F_{ST} is a measure of degree of genetic differentiation between subpopulations and it can be calculated by the formula given below:

$$F_{ST} = \frac{H_T - H_S}{H_T}$$

where;
H_S = average expected heterozygosity in the subpopulations (in the present study the breeds)
H_T = average heterozygosity of the total population (Nei and Kumar, 2000), which is when all the breeds are considered as one big population in the present study.

F_{ST} values range from 0 to 1 and higher F_{ST} values imply higher genetic differentiation between subpopulations. On the other hand, if gene flow between subpopulations is high genetic differentiation between subpopulations will be small. Therefore, F_{ST} value will also be small. The pair-wise F_{ST} values between four native Turkish cattle breeds were calculated with Arlequin package program (Excoffier *et al.*, 2005). The data were permutated for 1000 times in order to test the significance of the pair-wise F_{ST} values.

2.3.4 Principal Component Analysis (PCA)

In the present study, Principal Component Analysis (PCA) was used for visualizing population relationships (i.e. relative positions of populations) in 2 dimensional spaces. PCA was performed by using NTSYS (Numerical Taxonomy and Multi Variety Analysis System) package program (Rohlf, 2000). Two independent compound axes are synthesized from two variables and relative positions of the populations are visualized in the space generated by the PCA axes. The first axis explains the highest variation of all the data that can be accounted for by the compound axis; the second axis explains the next highest variation, (Jobling *et al.*, 2004).

CHAPTER 3

RESULTS

3.1 Results of the Laboratory Experiments

3.1.1 DNA extraction

The DNAs were extracted from the blood samples and then agarose gel electrophoresis was used in order to check the presence of DNA by using the procedures mentioned in the previous chapter. Figure 3.1 represents an example of the Markov-chain random walk algorithm described Guo and Thomson (1992). DNA check gel image visualized under UV light. Using such images, the presence of DNA bands and approximate quantity of DNAs were observed. Some samples did not contain any DNA (Figure 3.1, samples 30d and 38d) or revealed a weak product (Figure 3.1, samples 8d and 28d). The samples which did not have any DNA or had weak DNA were not used for PCR-RFLP assays and for those new DNAs were isolated. Some DNAs isolated from blood samples were highly concentrated (such as; Figure 3.1, samples 10d, 18d, 25d and 32d). Approximate concentrations were determined visually. These DNAs were diluted using TE buffer. Diluted DNAs were left overnight at room temperature. After, checking the concentration of diluted DNAs on 1% agarose gel, the DNA bands were compared with a known DNA concentration to decide how much DNA sample to use in PCR reaction mixture.

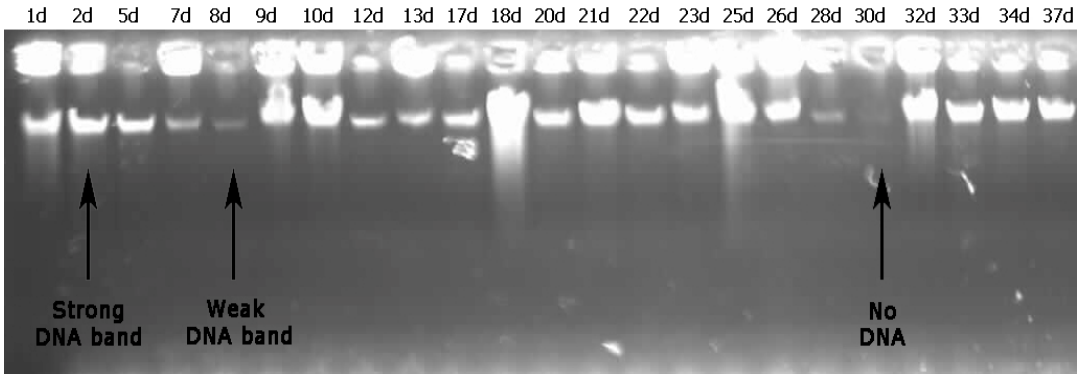

Figure 3.1 A check gel for total DNA extracts on 0.8% agarose gel. Those letters and numbers shown above the wells are identification codes of the samples used (such as, 1d: 1st sample of South Anatolian Red (d) breed).

After the preparation of DNA samples, amplification of three genes (*PRL*, *DGAT1* and *SLC35A3*) was carried out by using appropriate primers and PCR procedures. PCR products were checked on 2% agarose gel and photographed under UV light as will be shown in following sections (such as Figure 3.2). The quantity of DNA is important for the success of RFLP analysis. The gel images were used to decide which samples to be used in RFLP analysis and which ones to be re-amplified. Repeated samples which did not contain any PCR product at all (Figure 3.4, sample 1b) or resulted in weak products (Figure 3.6, sample 1c) were excluded from the analysis whereas the ones that have strong PCR products (Figure 3.2, sample 27a and 1c) were used in the RFLP analysis.

3.1.2 Genotyping

Genotyping of PCR products of *PRL*, *DGAT1* and *SLC35A3* genes were done by using PCR-RFLP method. The genotype of each individual for each studied locus was given in Appendix A.

3.1.2.1 *PRL* gene

The *PRL* gene was amplified using PCR amplification procedure mentioned in the previous chapter. Photograph representing PCR amplification results of *PRL* gene was given in Figure 3.2. After amplification, PCR products were digested overnight with *RsaI* restriction enzyme and run on 4% agarose gel. However, since 156-bp fragment of *PRL* gene region was very short and digestion fragments (74-bp and 82-bp) were very close to each other, agarose gel electrophoresis did not give clear results. After many trials in order to clarify the results by using agarose gel electrophoresis, it was decided to employ polyacrylamide gel electrophoresis (PAGE) procedure. Since, resolution power of PAGE is very high, eventually clear results were obtained by using this technique. Therefore polyacrylamide gel having higher resolving power was used instead of agarose gel. An example gel photograph showing the digestion products of *PRL* gene was given in Figure 3.3.

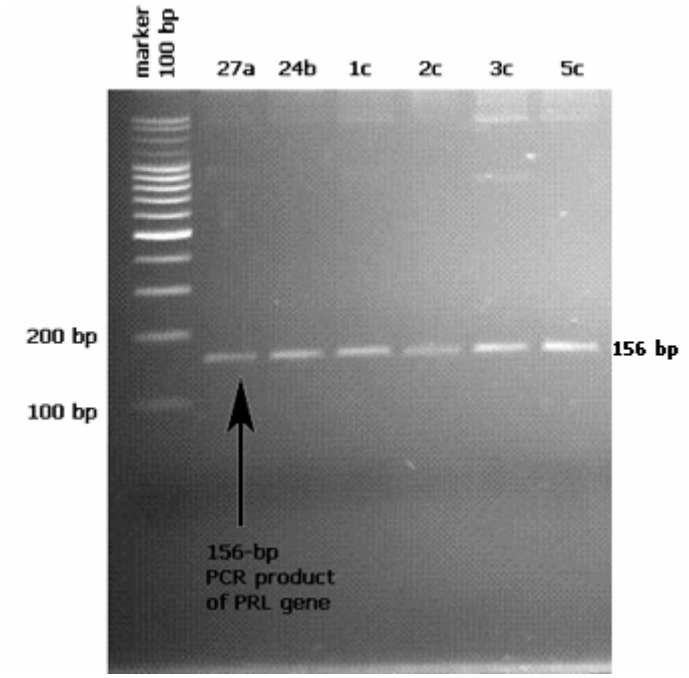

Figure 3.2 Photograph of PCR amplification products of *PRL* gene on agarose gel. Those letters and numbers shown above the wells are the identification codes of used samples (such as, 27a: 27th sample of Turkish Grey (a) breed).

The PCR product of the *PRL* gene is 156-bp and with the digestion of this product with *RsaI* restriction enzyme three different genotypes were obtained. The ones that have only 156-bp band (such as Figure 3.3, sample 24b) were genotyped as AA. Individuals that have 156-bp, 82-bp and 74-bp bands (such as Figure 3.3, sample 27a and 1c) were genotyped as AB and those having 74-bp and 82-bp bands (such as Figure 3.3, 3c) were genotyped as BB.

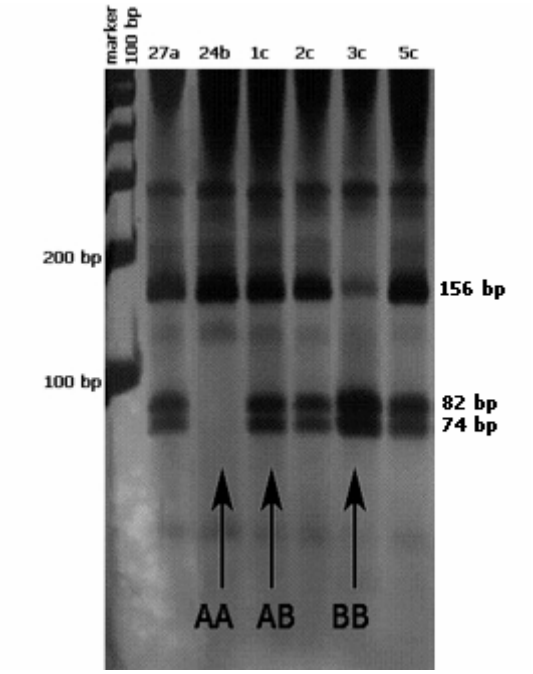

Figure 3.3 Photograph of digestion using products of PRL gene on polyacrylamide gel. Those letters and numbers shown above the wells are the identification codes of used samples (such as, 27a: 27th sample of Turkish Grey (a) breed).

3.1.2.2 *DGAT1* gene

A 411-bp of *DGAT1* gene was amplified by using the PCR amplification procedure given in "Materials and Method" section. Photograph representing PCR amplification results of *DGAT1* gene was given in Figure 3.4. Amplification products of *DGAT1* gene were digested overnight with *Cfr*I restriction enzyme.

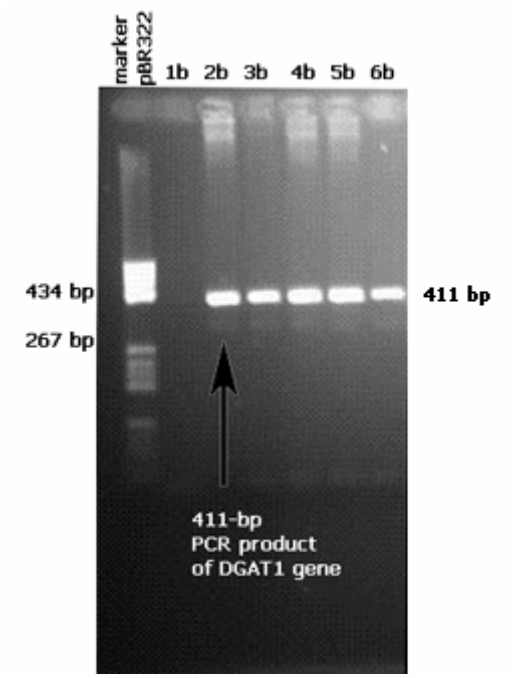

Figure 3.4 Photograph of PCR amplification products of DGAT1 gene on agarose gel. Those letters and numbers shown above the wells are the identification codes of used samples (such as, 2b: 2nd samples of East Anatolian Red (b) breed).

The digestion products were run on 4% agarose gel and results were visualized under UV light with ethidium bromide dye. An example gel photograph showing the digestion products of *DGAT1* gene was given in Figure 3.5.

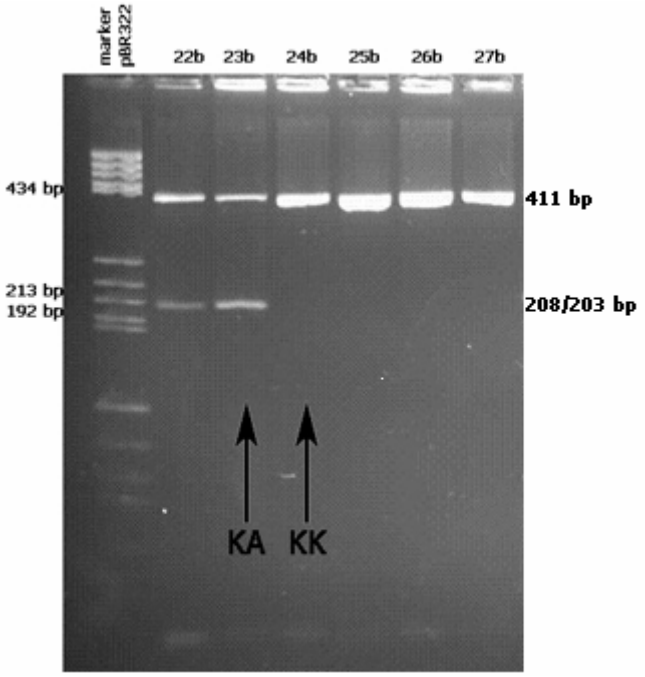

Figure 3.5 Photograph of digestion products of DGAT1 gene on agarose gel. Those letters and numbers shown above the wells are the identification codes of used samples (such as, 22b: 22nd samples of East Anatolian Red (b) breed).

The genotypes of some individuals for the *DGAT1* gene were given in Table 3.1. The PCR product of the *DGAT1* gene is 411 bp and with the digestion of this product with *CfrI* restriction enzyme three genotypes (KK, KA and AA) were expected. In the presented study, with the digestion of *DGAT1* gene only two genotypes, namely KK and KA were observed. The ones that have only 411-bp band (Figure 3.5, sample 24b) were genotyped as KK. Individuals that have 411-bp, 208-bp and 203-bp bands (Figure 3.5, sample 23b) were genotyped as KA.

3.1.2.3 *SLC35A3* gene

A 233-bp fragment of *SLC35A3* gene was amplified by the PCR amplification procedure. Photograph exhibiting amplification results of this gene was given in Figure 3.6. After that, the amplification products of *SLC35A3* gene were digested overnight with *Ecot22* restriction enzyme, the digestion products were run on 4% agarose gel and visualized under UV light with the ethidium bromide dye. Photograph An example gel photograph showing the digestion products of *SLC35A3* was given in Figure 3.7.

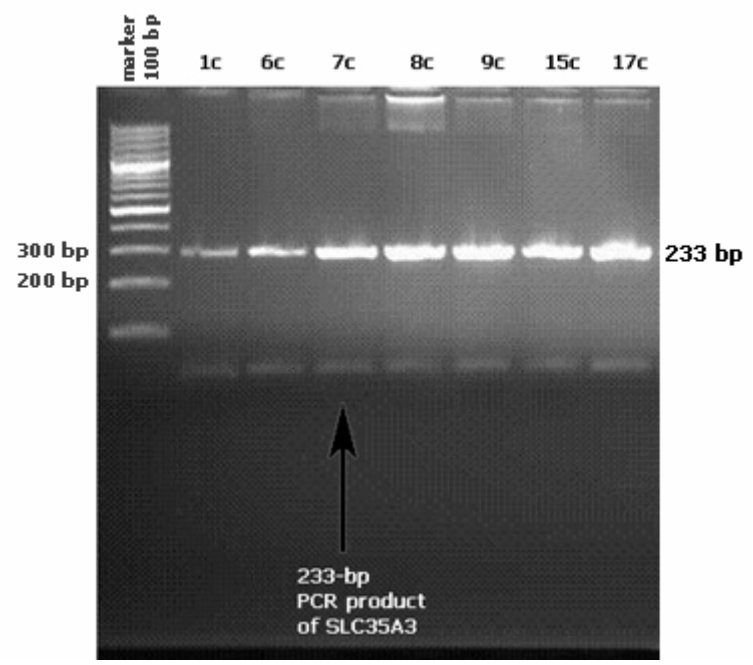

Figure 3.6 Photograph of PCR products of SLC35A3 (CVM disease related gene) on agarose gel. Those letters and numbers shown above the wells are the identification codes of used samples (Such as, 1c: First sample of Anatolian Black (c) breeds).

With the digestion of *SLC35A3* gene product with *Ecot22* restriction enzyme three different genotypes were expected as indicated in "Materials and Methods". However, in all analyzed populations only AA (healthy) genotype was observed (Figure 3.7).

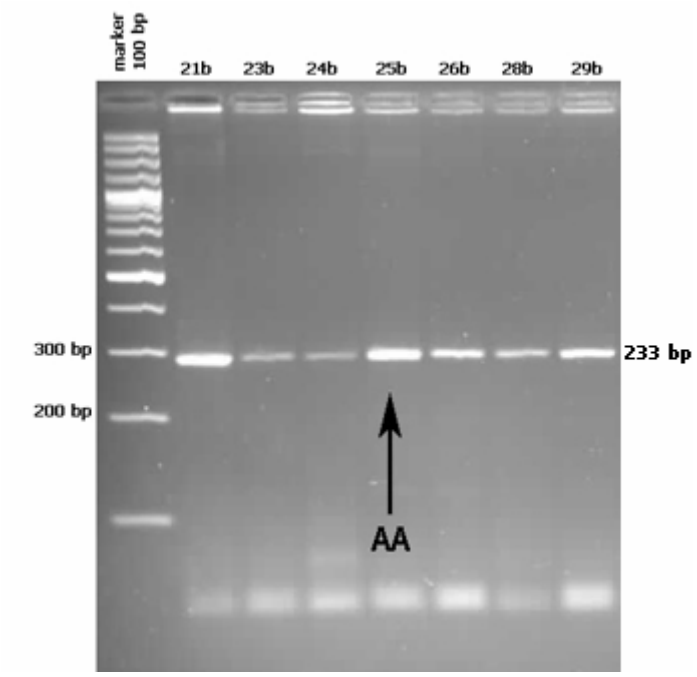

Figure 3.7 Photograph of digestion products of SLC35A3 on agarose gel. Those letters and numbers shown above the wells are the identification codes of used samples (such as, 21b: 21st sample of East Anatolian Red (b) breeds).

3.2 Results of Statistical Analyses

3.2.1 Allele frequencies, heterozygosities and Hardy-Weinberg equilibrium

Allele frequencies of five breeds (Turkish Grey, East Anatolian Red, Anatolian Black, South Anatolian Red, and Holstein) were calculated for *PRL*, *DGAT1* and *SLC35A3* genes based on the genotypes given in appendix A and results were given in Table 3.1 *PRL* and *DGAT1* were polymorphic whereas *SLC35A3* was monomorphic in all of the studied breeds. The observed allele frequencies together with observed heterozygosity and observed genotype numbers were given in Table 3.1 The presence of Hardy-Weinberg equilibrium was tested for all the studied breeds in two polymorphic loci (*PRL* and *DGAT1*). The expected genotype numbers, expected heterozygosities, and *p* values were also given in Table 3.1 Tests revealed that the two breeds, the Turkish Grey (p=0.0195*) and East Anatolian Red (p=0.0045**), were not in HWE for *PRL* gene.

In the present study, three genotypes (AA, AB and BB) were identified for the *PRL* gene. The highest frequency (0.8571) for A allele was detected in Holstein. On the other hand, when Turkish breeds were compared, the A allele was found with the highest frequency in South Anatolian Red (0.7558) and Turkish Grey (0.7000) breeds. It has the lowest frequency in Anatolian Black with the frequency of 0.5645. As expected by the high frequency of A allele in the Holstein breed, the most frequent genotype was the AA genotype (16/21=0.7619). BB genotype of *PRL* gene was found in Anatolian Black (3 samples) and South Anatolian Red (4 samples) breeds but BB genotype was not observed in Holstein, Turkish Grey and East Anatolian Red breed

In Turkish Grey and East Anatolian Red, the two breeds which were not in Hardy-Weinberg equilibrium, observed heterozygosities were higher than expected values.

In Turkish Grey breed, while number of expected heterozygosity was 18.9, number of observed heterozygositiy was 25. In East Anatolian Red population, while number of expected heterozygosity was 11, number of observed heterozygosity was 15.

The frequency of DGAT1 K allele was higher than the A allele in all of the studied breeds (Turkish Grey, East Anatolian Red, Anatolian Black, and South Anatolian Red). The A allele frequency was highest in East Anatolian Red with the frequency of 0.2205 whereas lowest in Anatolian Black (0.0789). For the studied breeds only KK and KA genotypes were identified

As indicated before, for the SLC35A3 gene all the studied breeds were monomorphic for the A allele. B allele, disease related allele, was not found in any of the individual in studied breeds. Hence, there was not even a single CVM disease carrier among the tested individuals and breeds.

Table 3.1 Table of Allele and genotype frequencies, observed and expected heterozygosities and p-values of four native Turkish breeds

Loci	Populations	N	Allele Frequencies		Genotypes						*p*	Observed Heterozygosity	Expected Heterozygosity
					Observed Number			Expected Number					
			A	**B**	**AA**	**AB**	**BB**	**AA**	**AB**	**BB**			
PRL	Turkish Grey	45	0.7000	0.3000	18	27	0	22.05	18.9	4.05	0.0046**	0.6000	0.4247
	East Anatolian Red	34	0.6617	0.3338	11	23	0	14.8867	15.0195	3.7883	0.0045**	0.6764	0.4543
	Anatolian Black	31	0.5645	0.4355	8	19	4	9.8784	15.242	5.8794	0.2765 (n.s.)	0.6129	0.4997
	South Anatolian Red	45	0.7558	0.2442	26	16	3	25.7055	16.6109	2.6834	0.6862 (n.s.)	0.3589	0.3862
	Holstein	21	0.8571	0.1428	16	5	0	15.4270	5.1405	0.4282	1.0000 (n.s.)	0.3333	0.2845
			K	**A**	**KK**	**KA**	**AA**	**KK**	**KA**	**AA**			
DGAT1	Turkish Grey	30	0.8500	0.1500	21	9	0	21.675	7.6500	0.6750	1.0000 (n.s.)	0.2500	0.2227
	East Anatolian Red	34	0.7794	0.2205	19	15	0	20.668	11.6863	1.6530	0.1679 (n.s.)	0.4411	0.3489
	Anatolian Black	20	0.9250	0.0789	17	3	0	18.6613	3.1973	0.1369	1.0000 (n.s.)	0.1500	0.1423
	South Anatolian Red	38	0.8289	0.1710	25	13	0	26.1088	10.7723	1.1111	0.5640 (n.s.)	0.3421	0.2873
	Holstein	0	-	-	-	-	-	-	-	-	-	-	-

Table 3.1 (continued)

			A	B	AA	AB	BB	AA	AB	BB			
SLC35A3	Turkish Grey	40	1	0	40	0	0	-	-	-	-	-	-
	East Anatolian Red	34	1	0	34	0	0	-	-	-	-	-	-
	Anatolian Black	32	1	0	32	0	0	-	-	-	-	-	-
	South Anatolian Red	37	1	0	37	0	0	-	-	-	-	-	-
	Holstein	21	1	0	21	0	0	-	-	-	-	-	-

ns - not significant >0.05, * < 0.05, ** < 0.01, *** < 0.001

3.2.2 FST Statistics

The F_{ST} index was used to analyze the degree of genetic differentiation among the studied breeds. F_{ST} analysis were performed first for each locus separately and then for multi-loci. Based on single-locus analysis, pairwise F_{ST} values for *PRL* and *DGAT1* were given in Table 3.2 and Table 3.3, respectively.

Table 3.2 F_{ST} values of five native Turkish cattle breeds based on PRL gene

POPULATIONS	***F_{ST}* Values**	
Turkish Grey-East Anatolian Red	-0.0096	ns
Turkish Grey-Anatolian Black	0.0260	ns
Turkish Grey-South Anatolian Red	-0.0073	ns
Turkish Grey-Holstein	0.0284	ns.
East Anatolian Red-Anatolian Black	0.0044	ns
East Anatolian Red-South Anatolian Red	0.0022	ns
East Anatolian Red-Holstein	0.0532	ns
Anatolian Black-South Anatolian Red	0.0557	*
Anatolian Black-Holstein	0.1341	**
South Anatolian Red-Holstein	0.0046	ns

ns-not significant, * < 0.05, ** < 0.01, *** < 0.001

F_{ST} analysis for *PRL* gene was indicated that Anatolian Black is significantly different than the South Anatolian Red (F_{ST}=0.0557*) and Holstein (F_{ST}=0.1341**) breeds (Table 3.2). On the other hand, no population differentiation between studied breeds (Turkish Grey, East Anatolian Red, Anatolian Black and South Anatolian Red) was obtained for *DGAT1* gene (Table 3.3).

Table 3.3 F_{ST} values of four native Turkish cattle breeds based on *DGAT1* gene

POPULATIONS	F_{ST} Values	
Turkish Grey-East Anatolian Red	0.0147	ns
Turkish Grey-Anatolian Black	-0.0082	ns
Turkish Grey-South Anatolian Red	-0.0074	ns
East Anatolian Red-Anatolian Black	0.0546	ns
East Anatolian Red-South Anatolian Red	-0.0062	ns
Anatolian Black-South Anatolian Red	0.0193	ns

ns-not significant, * < 0.05, ** < 0.01, *** < 0.001

For the multi-loci analysis F_{ST} test were repeated by considering the number of samples genotyped for at least two loci (Table 3.4). The number of samples used for the multi-loci analysis was given in Table 2.1. Significant differentiation was not observed between the subpopulations.

Table 3.4 F_{ST} values of four native Turkish cattle breeds based on two loci (PRL and SLC35A3).

POPULATIONS	F_{ST} Values	
Turkish Grey-East Anatolian Red	0.0098	ns
Turkish Grey-Anatolian Black	0.0169	ns
Turkish Grey-South Anatolian Red	-0.0036	ns
East Anatolian Red-Anatolian Black	0.0201	ns
East Anatolian Red-South Anatolian Red	-0.0085	ns
Anatolian Black-South Anatolian Red	0.0355	ns

ns-not significant, * < 0.05, ** < 0.01, *** < 0.001

3.2.3 Principal Component Analysis (PCA)

Based on the allele frequencies of, four native Turkish cattle breeds for *PRL* and *DGAT1*, Principal Component Analysis (PCA) was performed to examine and to visualize relative relatedness of populations in 2 dimensional spaces. Two axes of the PCA covers 100 % of the total variation. Image exhibiting relatedness of four populations in two dimensional space was given at Figure 3.8.

The equations of the two components (PC1 and PC2) of the PCA were as follows;
PCA= 0.9014 PRL-A - 0.9014 *DGAT1*-K
PCA = 0.4329 PRL-A + 0.4329 *DGAT1*-K

It can be deduced from the weightings of the variables (in the equations of the first and second components) that on the axes both genes contributed to the differentiation of the populations.

Furthermore, in the first axis because the signs of the coefficients are opposite one can say that the two genes contribute oppositely, whereas they act in the same direction in the second axis.

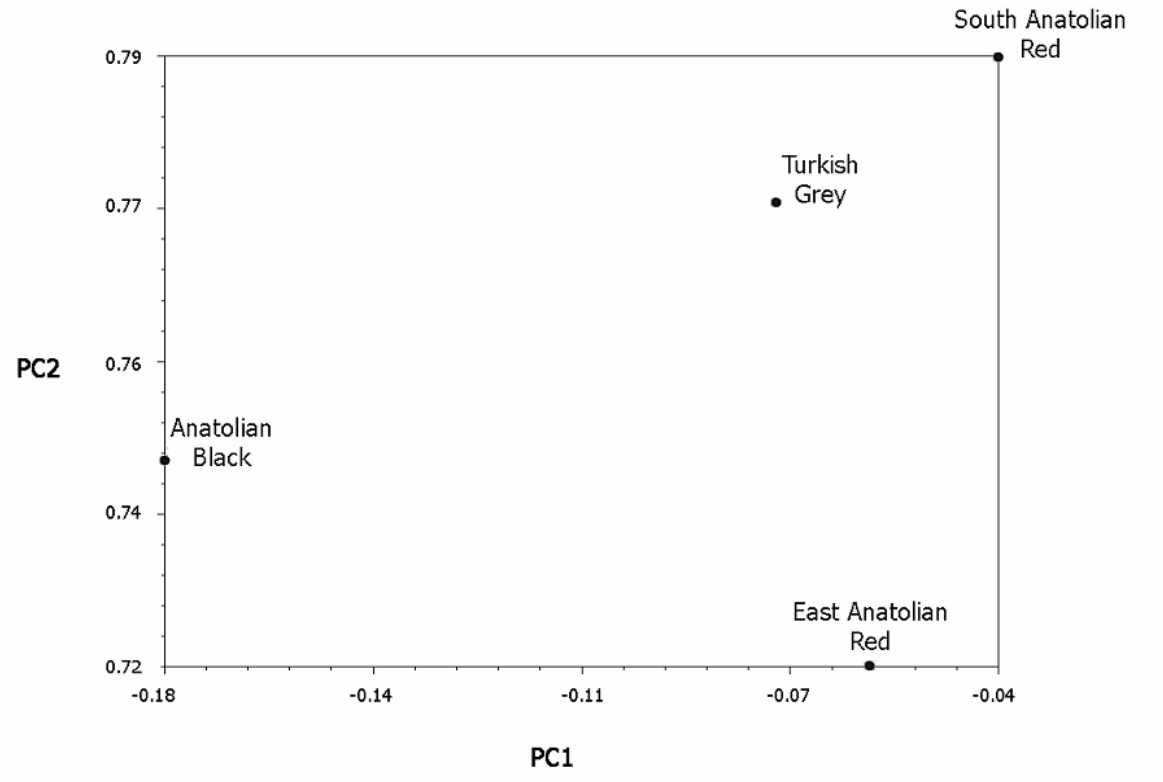

Figure 3.8 PCA of four native Turkish cattle populations.

As a result of the PCA first component (PC1) which explains the 81.4755% percentage of the genetic variation discriminates Anatolian Black from other three breeds (Turkish Grey, East Anatolian Red, and South Anatolian Red) clearly. Component two (PC2) of the PCA explaining the 18.5238% of the total variation divides native cattle breeds into two groups. Based on the PC2, South Anatolian Red and Turkish Grey breeds form one group and the Anatolian Black and East Anatolian Red forms another group.

CHAPTER 4

DISCUSSION

Milk is one of the most essential and complete nutrition sources for mammals. Some of the domesticated animals for instance cattle (Bradley *et al.,* 2006) were employed for milk production for the humankind in the last ten millennia. Milk can be used as row milk or can be converted into a variety of dairy products such as cheese and yoghurt by processing. Both the milk and its products have been subjected to different preferences. Sometimes, high milk yield with low fat content can be preferred for example in drinking milk; on the other hand milk with high fat content can be preferred in some yoghurt.

Breeds of cattle have differentiated gene pools. Differentiation was under the effects of selection (for example for the high milk yield), random genetic drift and environmental selection (natural selection). Nowadays, tracking the genes which are known to be associated with the properties of the milk one can identify the status of the breed in relation to the property under consideration. Then one may manage the breed based on the molecular information via marker assisted selection.

However, most of the traits in relation to properties of the milk are not controlled by a single gene. For example, in this study two of the genes (*PRL, DGAT1*) are known to be associated with the milk yield in cattle (see for example, Dybus, 2002 and Sanders *et al.,* 2006). If management is going to be carried out based on one gene it

might be inefficient. Furthermore, while focusing on the selection of one locus other loci contributing to the trait might lose their integrity and hence, attempt of management may be a complete failure in the result. Therefore, all the information based on many genes must be collected first. Their possible interrelations should be analysed and then must be employed in the management. For the milk yield for example as well as A allele of *PRL* and A allele of *DGAT1,* A allele of *β-laktoglobulin (*Tsiaras *et al.,*2005; Daniela *et a.,l* 2005) is also known to be related with the milk yield in cattle.

Another issue is when focusing on one property such as milk yield of milk, gene pool of the breeds might suffer from; i) erosion of gene diversity and ii) gene pollution. While the former is unavoidable, the latter, consequence of introgression (for example for high milk yield) or hitchhiking is something one can control and avoid. The affect of latter can be so serious that it may sweep the gain from selection. One of such gene could be *SLC35A3*. While, trying to increase the milk yield in Turkey sperm of elite Holstein bulls were heavily used to fertilize the native cows, this may introduce and increase the frequency of CVM carriers. It was observed in several studies that not only still born are the problem but also the carriers and hence the economy is adversely affected by CVM (Nielsen *et al.*, 2002; Agerholm *et al.*, 2004; Berglund *et al.*, 2004 and Mallher *et al.*, 2005).

Milk and milk products, both their quantity and quality are important for the economy of the country. In the present study, as well as the disease related allele of *SLC35A3* gene, allele and genotype frequencies of two genes related with the milk yield and fat content of the milk were examined in Turkish native cattle breeds and in a sample of Holstein breed for *PRL* and *DGAT1* loci.

4.1 PRL gene in Turkish breeds

Observed range for the A allele of *PRL* locus was 0.61 in German Black and White (Khatami *et al.,* 2005), 0.97 in Lithuanian White Backed (Miceikiene *et al.,* 2006) and the frequencies were more often towards the upper end of the range. In Holstein breed which is a well known breed selected for high milk yield A allele frequency of *PRL* gene was 0.95 (Chung *et al.,* 1996 and Chrenek *et al.,* 1998 referred by Miceikiene *et al.,* 2006).

There was no previous observation on *PRL* gene frequency in Turkish native breeds. In the present study the observed range being 0.56 (Anatolian Black) 0.76 (South Anatolian Red) is narrow and the values are relatively low. All of the allele frequencies were calculated based on sample sizes, n=31-45 for the native breeds. Hence, allele frequencies must be quite reliable.

Whereas in Holstein sample A allele frequency was high (0.8571) in accordance with the expectations from a breed with high milk yield. Yet, even the value observed in Turkey (0.85) was not as high as some values observed (0.95) in other Holstein samples before (Chung *et al.,* 1996 and Chrenek *et al.,* 1998 referred by Miceikiene *et al.,* 2006). Anatolian Black (AB) displayed unexpectedly low value; lower than all of the previous observations in the literature. In Turkey, with respect to *PRL* gene, AB was significantly different than that from South Anatolian Red ($p<0.05$). Furthermore, it was more significantly ($p<0.01$) different than that of Holstein sample.

In Figure 4.1 Milk production per individual was shown for different countries (Milk and milk products in theEuropean Union, 2006). If we consider average milk yield/individual during the lactation period in Turkey (http://www.tarimsal.gov.tr) the maximum value is 1875.36 for the South Eastern Anatolian Red. Maximum yield of Turkish native cattle breeds seems to be half of the country average of

Lithuania (country with the minimum milk production among the European Countries). Ranking of the Turkish native breeds in relation to their milk yield (kg) is; South Anatolian Red (1875 kg), Turkish Grey (1096 kg) Anatolian Black (1062 kg), East Anatolian Red (939 kg) (http://www.tarimsal.gov.tr). Hence, observation (http://www.tarimsal.gov.tr) that low A frequency and low milk yield in Turkish native breeds fits well. Rank in the A frequency of *PRL* is South Anatolian Red, Turkish Grey East Anatolian Red Anatolian Black is not exactly parallel to their milk yield. Nevertheless, AB has the minimum milk yield in Turkish native breeds and has the lowest A allele frequency of PRL gene and it is significantly different in A compared to that of the SAR which has the maximum milk yield in Turkey. Of course, milk yield is affected by the environmental conditions heavily. However results of the present study suggests that perhaps genetic compound; low *PRL* A allele frequency may also be playing role in low milk yield of our native breeds.

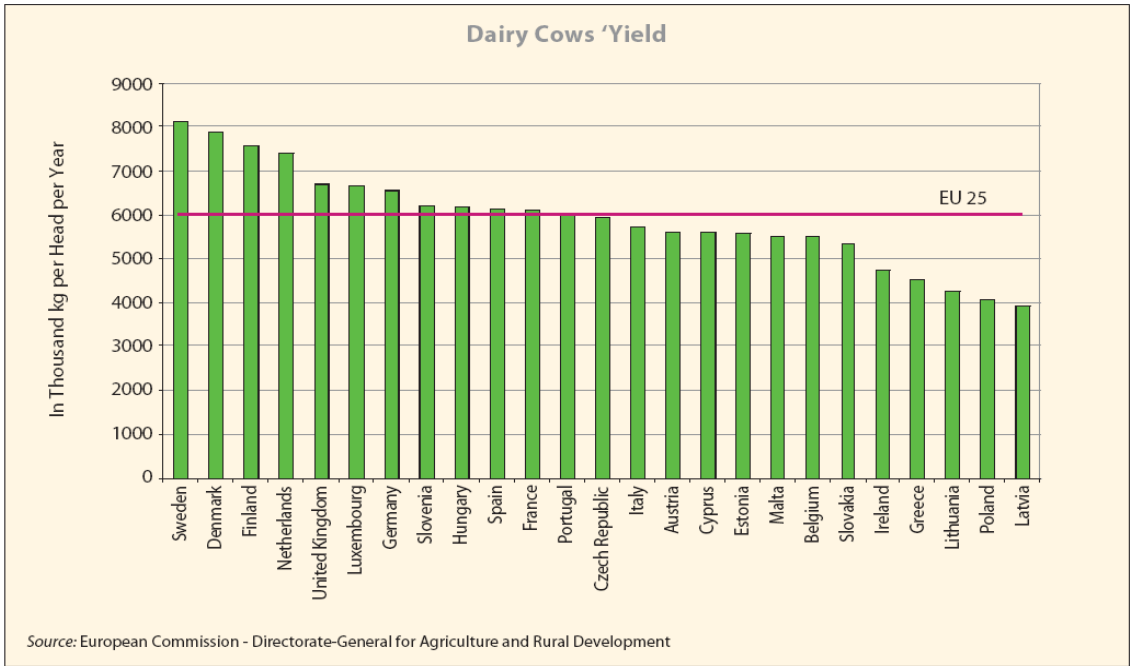

Figure 4.1 Average milk production for per head per year in EU countries

It is observed that within the breed BB genotype cows had lower milk yield and higher fat content than those with AA and AB genotypes (Khatami *et. al.,* 2005). With respect to fat contents, lowest A and hence highest frequency of B is found in Anatolian Black and indeed the highest fat content (4.5%) is associated with this breed (http://www.tarimsal.gov.tr). Similarly lowest B lowest fat content (3.2%) is seen in South Anatolian Red. However, for the other two breeds possessing intermediate B frequencies and intermediate fat contents association is not perfect. Another, observation to be discussed in relation to *PRL* locus is, it exhibited deviation in Hardy –Weinberg equilibrium in two of the breeds (Turkish Grey and East Anatolian Red) out of 5 considered, observed heterozygosities were higher than expected values. Since there was no further deviation from HW expectations in relation to *DGAT1* gene since in both of the breeds heterozygotes exceeded the expected values it can be suggested that by chance we are observing perhaps excess of the results of AA males X BB females type of crosses.

In relation to methodology, RFLP analysis of *PRL* gene was planned to be done by the procedure given by Dybus (2002). However, this procedure was slightly modified in the present study. Because digestion products of *PRL* gene were very short (74 bp and 82 bp) and similar in size, for visualization of the bands polyacrylamide gel instead of agarose was preferred.

4.2 DGAT1 Gene in Turkish breeds

More than one gene has effect on milk composition and milk yield. The second gene affecting these two properties of milk is *DGAT1*. *DGAT1* K allele seems to be parallel to the B allele of *PRL* gene; it is associated with high fat content of the milk. However, while high K frequency was observed in beef breeds in the dairy cattle everything from very low levels to very high K allele frequencies were observed (Kaupe *et al.,* 2003). The highest K frequencies were observed in Zebu breeds

(88%-92%) and Turkish native breeds exhibited intermediate values (21%-38%) in a previous study (Kaupe *et al.*, 2003).

However, in the present study observed frequencies of K allele (0.78%-0.93%) are higher than those observed before for the native Turkish breeds and they are in the range of Zebu breeds. One reason for the discrepancy of the results could be the difference in the methods. However, methods are the same. Second reason could be the difference in the samples. For another locus in cattle, *β-laktoglobulin*, presumably based on the same samples of the breeds again differences in the allele frequencies in relation to the samples of the same breeds were observed for the *β-casein, β-lactoglobulin and κ-casein* genes (Togan *et al.*, 2007: Jann *et al.*, 2004). These observations emphasize the importance of sampling in the breeds. In sampling a breed, to represent its gene pool, maximum possible geographic area must be covered and very few, 1-3 individuals must be sampled/herd as suggested by a 5th frame EU project (Econogene Project, 2005).

Since, sampling strategies were not given (previous studies) or known to be different (present study) than the one suggested by Econogene Project (Econoge Consortiom, 2005) probably none of the samples; neither the one in the present study nor the one in the previous studies can be considered as the best representatives of the breeds.

The rank in K allele frequency is Anatolian Black>Turkish Grey> South Anatolian Red> East Anatolian Red. Whereas rank in fat content is follows: Anatolian Black > Turkish Grey> East Anatolian Red> South Anatolian Red. Hence, an association, although not perfect, between the two variables was observed.

What could be the reason of high K frequency? Several explanations can be suggested. Native Turkish breeds were never subjected to systematic selection for high milk yield. They are assumed to be close relatives of the early domesticated cattle. *DGAT1* is known to be present in Zebu cattle (Winter *et al.*, 2002 Kaupe *et al.,* 2003) and Water Buffalo (Winter *et al.,* 2002), hence it must evolutionary be an

old variant. Perhaps, at the first domestics, K allele was frequent. However, perhaps in western European dairy breeds during the selection for the high milk yield allele frequency was reduced. Alternatively, it is known that, in Anatolia native breeds have contribution from Zebu (Loftus *et al.*, 1999; Freeman *et al.*, 2005 and Özkan, 2005) and therefore allele frequency is high.

4.3 PRL and DGAT1 in Turkish breeds

Principal Component Analysis summarized the results of two genes in 4 breeds on two axes. It can be seen that the first axis accounting the 81.4755% percentage of the genetic variation discriminates Anatolian Black from other three breeds (Turkish Grey, East Anatolian Red, and South Anatolian Red). Component two (PC2) of the PCA explaining the 18.5238% of the total variation seemed to divide native cattle breeds into two groups: South Anatolian Red and Turkish Grey breeds form one group and the Anatolian Black and East Anatolian Red forms another group.

However, none of the pairwise Fst values were found to be significantly different from each other and hence none of the breeds are significantly different from each other based on the *PRL and DGAT1* loci when studied jointly. Yet, it seems that allele frequencies provide an ordering for the breeds with respect to the first axis Anatolian Black (fat content: 4.55%), Turkish Grey (fat content: 3.93%), Eastern Anatolian Red (fat content: 3.45%), South Anatolian Red (fat content: 3.2%) accounts a perfect correlation with their fat content (Figure.4.2.). Second axis, displays another order South Anatolian Red (average milk yield: 1875kg), Turkish Grey (average milk yield: 1096 kg), Anatolian Black (average milk yield: 1062kg), East Anatolian red (average milk yield: 939kg) which again is perfectly correlated with the reported milk yields (Figure 4.2.). Observed perfect ordering is remarkable. It emphasizes that both of the genes are affecting the milk yield and fat content of the milk in the breeds. Furthermore, it suggests a frame about how milk yield and fat content will vary in accordance with the allele frequencies of the two genes. First axis clearly shows that the fat content increases with K of *DGAT1* and decreases

with A of *PRL*. Because the coefficients are both high and because the axis account high percent of the total variability (81.4755%) it can be suggested that each generates a quick response in fat. The most interestingly, the second axis, however, is generated by the positive contribution of both A and K. So while increasing K, not only fat content, but also milk yield is increased The observed wide range of K in dairy cattle (Kaupe *et al.,* 2003) might also be explained by their parallel effects in milk yield. In the absence of molecular markers humans might have selected some breeds for high K and some for high A, both for the purpose of high milk yield. Anatolian breeds might be those in the line of high K breeds. But, perhaps A related milk yield is higher than that of K related milk yield.

Again, one may anticipate that, a heavy, A related milk yield also effected the gene pool such that breed became more vulnerable to the extremes of environmental conditions as was the case for economically important breeds such as Holstein breed.

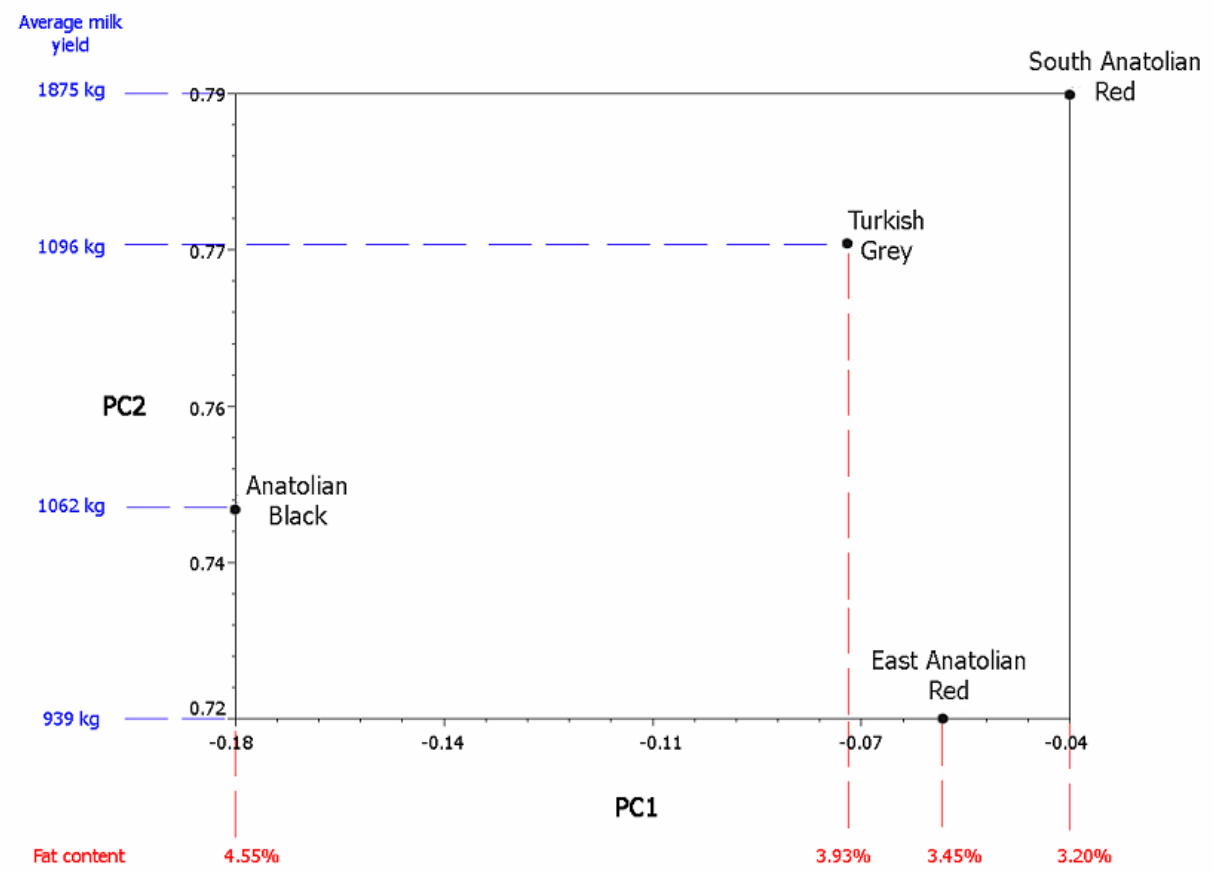

Figure 4.2 PCA result of PRL and DGAT1 genes

4.4 CVM Disease (Complex Vertebral Malformation)

The spread of recessive genetic defect in domestic animal populations may lead to very important economic losses and may impair the efficiency of genetic improvement programmes. One such genetic defect known as Complex Vertebral Malformation (CVM) was first observed in Denmark (Agerholm *et al.,* 2001), and recognized as a problem in the United States (Duncan *et al.,* 2001), the United

Kingdom (Revell, 2001), the Netherlands (Wouda *et al.,* 2000) and Japan (Nagahata *et al.,* 2002) referred by (Thomsen *et al.,* 2005). A point mutation from G to T at nucleotide position 559 of bovine solute carrier family 35 member of 3 *(SLC35A3)* caused an abnormal nucleotide-sugar transport into the Golgi apparatus (Thomsen *et al.*, 2005). The carrier elite bull, due to superior lactation performance of his daughters was extensively used and hence the wide spread problem was caused (Thomsen *et al.*, 2005).

In the present study possible introduction of the defect to Turkish native breeds was tested. The defect was not observed in neither the 21 elite bulls of Cattle Breeders Association of Turkey nor in any of the 143 individuals of four native Turkish cattle (South Anatolian Red, East Anatolian Red, Anatolian Black and Turkish Grey) breeds.

As a conclusion, in the present study, for the economically important 3 cattle genes (*PRL, DGAT1, SLC35A3*) an easy, RFLP based molecular genotyping was repeated and established in our laboratory, with minor modifications in *DGAT1* locus. Observed low milk yield for our native breeds correlated with low frequency of A allele in *PRL* locus. Similarly, K allele frequency of *DGAT1* locus was parallel to the fat content of the milks of Turkish native breeds. Yet, when both of the genes were considered jointly it was suggested that both A and K alleles of the genes have contributions in milk yield. It is known that *PRL* and *DGAT1* are only the two genes related with the milk yield. Other genes must also be examined. PCA seems to be a promising easy method that can be used to reveal pattern of cooperative effect of many genes in milk yield.

Based on the results of the present study, in order to improve the milk yield of Turkish native breeds perhaps, A allele can tried to be enriched in dairy cattle. However, it must be known that enrichment might be at the expense of some genetic variability, which perhaps is related with the genes confirming the local adaptation.

Finally, elite bulls and the Turkish native cattle breeds in this study seemed to be free from the CVM.

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APPENDIX A

Table 1.

BREED NAME	**GENE 1**	**GENE 2**	**GENE 3**
TURKISH GREY	***PRL***	***DGAT1***	***SLC35A3***
GREY STEP 1	AA	KK	AA
GREY STEP 2	AB	-	AA
GREY STEP 3	AA	KK	AA
GREY STEP 4	AA	KK	AA
GREY STEP 5	AB	KK	AA
GREY STEP 6	AB	KK	AA
GREY STEP 7	AA	KK	AA
GREY STEP 8	AB	KK	AA
GREY STEP 9	AB	-	AA
GREY STEP 10	AB	KK	AA
GREY STEP 11	AA	-	AA
GREY STEP 12	AB	KA	AA
GREY STEP 13	AB	-	AA
GREY STEP 14	AA	KA	AA
GREY STEP 15	AA	KK	AA
GREY STEP 16	AA	-	AA
GREY STEP 17	AA	KK	AA
GREY STEP 18	AB	KK	AA
GREY STEP 19	AB	KK	AA
GREY STEP 20	AA	KA	AA
GREY STEP 21	AB	KK	AA
GREY STEP 22	AA	KA	AA
GREY STEP 23	AA	KA	AA

Table 1. (continued)

GREY STEP 24	AB	-	-
GREY STEP 25	AA	KK	AA
GREY STEP 26	AB	KK	AA
GREY STEP 27	-	KA	AA
GREY STEP 28	AA	KA	AA
GREY STEP 29	AA	KA	AA
GREY STEP 30	AB	KK	AA
GREY STEP 31	AB	-	AA
GREY STEP 32	AB	-	-
GREY STEP 33	AB	KK	AA
GREY STEP 34	AB	-	AA
GREY STEP 35	AA	KK	AA
GREY STEP 36	AB	-	-
GREY STEP 37	AB	KK	AA
GREY STEP 38	AB	KK	AA
GREY STEP 39	AB	-	AA
GREY STEP 40	AB	-	-
GREY STEP 41	AB	KK	AA
GREY STEP 42	AB	-	AA
GREY STEP 43	AB	-	AA
GREY STEP 44	AB	-	-
GREY STEP 45	AA	-	-
GREY STEP 46	AA	-	AA

Table 2.

BREED NAME	GENE 1	GENE 2	GENE 3
EAST ANATOLIAN RED	***PRL***	***DGAT1***	***SLC35A3***
EAST ANATOLIAN RED 2	AB	KA	AA
EAST ANATOLIAN RED 3	AB	KA	AA
EAST ANATOLIAN RED 4	AA	KA	AA
EAST ANATOLIAN RED 5	AB	KK	AA
EAST ANATOLIAN RED 6	AA	KK	AA
EAST ANATOLIAN RED 7	AB	KK	AA
EAST ANATOLIAN RED 8	AB	KK	AA
EAST ANATOLIAN RED 18	AA	KA	AA
EAST ANATOLIAN RED 19	AB	KA	AA
EAST ANATOLIAN RED 20	AB	KA	AA
EAST ANATOLIAN RED 21	AA	KA	AA
EAST ANATOLIAN RED 22	AB	KK	AA
EAST ANATOLIAN RED 23	AA	KK	AA
EAST ANATOLIAN RED 24	AB	KK	AA
EAST ANATOLIAN RED 25	AB	KK	AA
EAST ANATOLIAN RED 26	AB	KK	AA
EAST ANATOLIAN RED 27	AB	KK	AA
EAST ANATOLIAN RED 28	AB	KA	AA
EAST ANATOLIAN RED 29	AB	KK	AA
EAST ANATOLIAN RED 30	AA	KA	AA
EAST ANATOLIAN RED 31	AA	KK	AA
EAST ANATOLIAN RED 32	AA	KK	AA
EAST ANATOLIAN RED 33	AA	KA	AA
EAST ANATOLIAN RED 34	AB	KK	AA
EAST ANATOLIAN RED 35	AA	KK	AA

Table 3.

BREED NAME	**GENE 1**	**GENE 2**	**GENE 3**
NATIVE BLACK	***PRL***	***DGAT1***	***SLC35A3***
NATIVE BLACK 1	AB	KK	AA
NATIVE BLACK 2	AB	KK	AA
NATIVE BLACK 3	BB	KK	AA
NATIVE BLACK 4	AA	KK	AA
NATIVE BLACK 5	AB	KK	AA
NATIVE BLACK 6	AB	-	AA
NATIVE BALCK 7	AA	KK	AA
NATIVE BLACK 8	AB	KK	AA
NATIVE BLACK 9	BB	KK	AA
NATIVE BLACK 10	AA	-	-
NATIVE BLACK 13	AA	KK	AA
NATIVE BLACK 14	AA	KK	AA
NATIVE BLACK 15	AB	KA	AA
NATIVE BLACK 16		KK	
NATIVE BLACK 17	AB	KK	AA
NATIVE BALCK 18	AB	KK	AA
NATIVE BLACK 19	AB	-	AA
NATIVE BALCK 20	AA	-	-
NATIVE BALCK 21	AB	KA	AA
NATIVE BLACK 22	AA	-	AA
NATIVE BLACK 24	AB	-	AA
NATIVE BLACK 25	-	KA	AA
NATIVE BALCK 26	AA	KK	AA
NATIVE BLACK 27	AB	-	AA
NATIVE BLACK 28	AB	-	-

Table 3. (continued)

NATIVE BLACK 29	AB	-	AA
NATIVE BLACK 30	AB	-	AA
NATIVE BLACK 31	-	-	AA
NATIVE BLACK 32	AB	KK	AA
NATIVE BLACK 38	AB	-	-
NATIVE BLACK 39	BB	-	-
NATIVE BLACK 40	AB	KK	AA
NATIVE BLACK 41	AB	-	-
NATIVE BLACK 42	BB	KK	AA

Table 4.

BREED NAME	GENE 1	GENE 2	GENE 3
SOUTH ANATOLIAN RED	***PRL***	***DGAT1***	***SLC35A3***
SOUTH ANATOLIAN RED1	AA	AA	AA
SOUTH ANATOLIAN RED2	AA	AA	AA
SOUTH ANATOLIAN RED3	AA	-	AA
SOUTH ANATOLIAN RED4	AA	AA	AA
SOUTH ANATOLIAN RED5	AB	AA	AA
SOUTH ANATOLIAN RED6	AB	-	AA
SOUTH ANATOLIAN RED7	BB	AA	AA
SOUTH ANATOLIAN RED8	-	-	AA
SOUTH ANATOLIAN RED9	AA	AB	AA
SOUTH ANATOLIAN RED10	AB	AA	AA
SOUTH ANATOLIAN RED11	AB	AA	AA
SOUTH ANATOLIAN RED12	AB	AB	AA
SOUTH ANATOLIAN RED13	AB	AA	AA
SOUTH ANATOLIAN RED14	AA	-	AA
SOUTH ANATOLIAN RED15	AA	AB	AA
SOUTH ANATOLIAN RED16	AB	AB	-
SOUTH ANATOLIAN RED17	AA	AA	AA
SOUTH ANATOLIAN RED18	AA	AB	AA
SOUTH ANATOLIAN RED19	AB	AA	-
SOUTH ANATOLIAN RED20	AA	KA	AA
SOUTH ANATOLIAN RED21	AB	AA	AA
SOUTH ANATOLIAN RED22	AA	AB	AA
SOUTH ANATOLIAN RED23	AA	AB	AA
SOUTH ANATOLIAN RED24	AA	AB	AA
SOUTH ANATOLIAN RED25	AA	AB	AA
SOUTH ANATOLIAN RED26	AA	AA	AA
SOUTH ANATOLIAN RED27	AA	-	-
SOUTH ANATOLIAN RED28	AA	AA	AA
SOUTH ANATOLIAN RED29	AB	AA	AA
SOUTH ANATOLIAN RED30	-	AA	AA
SOUTH ANATOLIAN RED31	AB	-	-

SOUTH ANATOLIAN RED32	AB	AB	AA

Table 4. (continued)

SOUTH ANATOLIAN RED33	AA	AA	AA
SOUTH ANATOLIAN RED34	AA	AB	AA
SOUTH ANATOLIAN RED35	AA	-	-
SOUTH ANATOLIAN RED36	AB	AA	AA
SOUTH ANATOLIAN RED37	-	AA	AA
SOUTH ANATOLIAN RED38	AB	-	AA
SOUTH ANATOLIAN RED39	BB	AB	AA
SOUTH ANATOLIAN RED40	AA	AA	AA
SOUTH ANATOLIAN RED41	BB	AA	AA
SOUTH ANATOLIAN RED42	AA	-	AA
SOUTH ANATOLIAN RED43	-	AA	AA
SOUTH ANATOLIAN RED44	-	AA	AA
SOUTH ANATOLIAN RED45	-	AA	AA
SOUTH ANATOLIAN RED46	-	AA	AA
SOUTH ANATOLIAN RED47	-	AA	AA

Table 5.

BREED NAME	GENE 1	GENE 2	GENE 3
HOLSTEIN	***PRL***	***DGAT1***	***SLC35A3***
HOLSTEIN 1	AA	-	AA
HOLSTEIN2	AB	-	AA
HOLSTEIN 3	AB	-	AA
HOLSTEIN 4	AA	-	AA
HOLSTEIN 5	AA	-	AA
HOLSTEIN 6	AA	-	AA
HOLSTEIN 7	AB	-	AA
HOLSTEIN 8	AA	-	AA
HOLSTEIN 9	AA	-	AA
HOLSTEIN 10	AA	-	AA
HOLSTEIN 11	AA	-	AA
HOLSTEIN 12	AA	-	AA
HOLSTEIN 13	AB	-	AA
HOLSTEIN 14	AA	-	AA
HOLSTEIN 15	AB	-	AA
HOLSTEIN 16	AA	-	AA
HOLSTEIN 17	AA	-	AA
HOLSTEIN 18	AA	-	AA
HOLSTEIN 19	AB	-	AA
HOLSTEIN 20	AA	-	AA
HOLSTEIN 21	AB	-	AA