

1 DGAT1-*Cfr*I polymorphisms on milk yield were observed ($P>0.05$). It would be
2 beneficial to increase the number of studies that determine the variation of existing
3 genes and investigated the effects of these polymorphisms on milk yield characteristics
4 in Holstein cattle. Thus, obtained results can be used as a selection criterion in marker-
5 assisted selection programs.

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7 **Key words:** Holstein; genetic polymorphism; milk protein genes; PCR-RFLP

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9 **1. Introduction**

10 Animal products are an indispensable food source for humans due to their biological
11 properties. In this context, animal production, which is the source of foods such as meat,
12 milk and eggs, has an important place in the agricultural sector across the world. Turkey
13 is suitable for almost all kinds of animal breeding in which cattle breeding are the most
14 important part of livestock production in the country cattle breeds in Turkey meet
15 approximately 88% of red meat and 90% of milk production¹. Holstein breed, which has
16 the ability to adapt to a wide range of climatic zones, is mainly preferred by farmers in
17 Turkey as in the world, therefore the number of pure and hybrid has increased rapidly.

18 Milk proteins contain all of the nine essential amino acids necessary for humans. Cow's
19 milk contains 3-5% proteins, which of casein consist of approximately 70-80% of this
20 protein, whey protein makes up the remaining 20-30%. Casein is composed of four
21 different types known as α_{s1} -casein, α_{s2} -casein, β -casein and κ -casein. Determination of

¹TUIK, Türkiye İstatistik Kurumu (2019). Website <http://www.tuik.gov.tr> [accessed 05.05.2021].

1 milk protein variants in different cattle breeds is an important application aiming to
2 avoid the increase of mutations that negatively affect specifically cheese making.

3 Located on bovine chromosome 6, the κ -casein gene² encodes milk protein important
4 for the structure and stability of casein micelles. Additionally, κ -casein is a very
5 important protein for cheese making. The rennin enzyme necessary to make milk
6 coagulate and curdle destabilizes this protein. In the light of previous studies, the κ -
7 casein gene is seen as a strong candidate gene for MAS in dairy and beef cattle [1-13].

8 Whey proteins make up the soluble fraction and they are composed of several different
9 proteins, the most important of which are α -lactoalbumin and β -lactoglobulin. Locating
10 on bovine chromosome 11³, β -lactoglobulin is one of the major whey proteins of
11 ruminant species. The biological functions of this protein are still unclear; however, it
12 could have a role in the metabolism of phosphate in the mammary gland, on the
13 transport of retinol and fatty acids within the gut [14]. Because of its potential for use in
14 genetic selection of the β -lactoglobulin gene polymorphism, several studies have been
15 carried out to identify genetic variation and frequencies of their variants in different
16 cattle breeds [3-6,9,10,15-18].

17 Prolactin, known as a lactogenic hormone, is essential for the initiation and maintenance
18 of lactation. It is mainly responsible promoting the synthesis and secretion of milk

²NCBI, National center for biotechnology information (2021). Website
https://www.ncbi.nlm.nih.gov/nuccore/AJ849456.1#goto54114662_0. [accessed
07.12.2021].

³NCBI, National center for biotechnology information (2021). Website
https://www.ncbi.nlm.nih.gov/nuccore/X14710.1#goto127_0 [accessed 07.12.2021].

1 proteins, lactose, lipids and all other major components of milk [19]. The bovine
2 prolactin gene is located on chromosome 23⁴. Allelic variation in the regulatory
3 sequences of these genes could be of interest due to their possible direct or indirect
4 effects on milk production and growth performance [19]. The prolactin gene, which has
5 an important regulatory function in milk production, is an important candidate gene for
6 QTL. Therefore, in many studies, the relationships between prolactin polymorphism and
7 milk yield characteristics were studied [9-26].

8 The gene of acylCoA-diacylglycerol acyltransferase1 (DGAT1)⁵ is located in the
9 centromeric region of bovine chromosome 14 and the locus of DGAT1 was identified as
10 that underlying the quantitative trait locus for milk production parameters. The DGAT1
11 is a positional candidate gene for milk fat percentage with K232A substitution
12 associated with the higher fat percentage in Bos Taurus [27]. DGAT1 gene
13 polymorphism and the effect of this polymorphism on milk yield characteristics were
14 investigated in many studies [21,27-33].

15 It is normal to observe different variants in genome due to genetic variations within and
16 between various species. It is possible that the presence and frequencies of alleles
17 encoding milk protein are different among individuals belonging to different cattle

⁴NCBI, National center for biotechnology information (2021). Website
[https://www.ncbi.nlm.nih.gov/nuccore/
NM_173953.2#goto46810276_0](https://www.ncbi.nlm.nih.gov/nuccore/NM_173953.2#goto46810276_0) [accessed
07.12.2021].

⁵NCBI, National center for biotechnology information (2021). Website
https://www.ncbi.nlm.nih.gov/nuccore/AY065621.1#goto18642597_0 [accessed
07.12.2021].

1 breeds. Their polymorphisms partly explain the genetic variance and improve the
2 estimated breeding value. Therefore, milk protein genes could be useful as genetic
3 markers for additional selection criteria in dairy cattle breeding.

4 The aim of this study was to determine polymorphisms in κ -casein, β -lactoglobulin,
5 prolactin and DGAT1 loci and their effects on milk yields in Holstein cows registered to
6 the Cattle Breeders' Association of Turkey in Antalya province.

7 **2. Materials and methods**

8 A total of 517 blood samples were randomly collected from seven different herds of
9 Holstein cows raised in Antalya province, Turkey. According to rules of the law
10 approving ethical handling of animals, the blood samples were taken from jugular vena
11 into tubes with 5% EDTA and stored at 4 °C until DNA extraction was performed.
12 Genomic DNA was extracted from blood using the GenElute Blood Genomic DNA kit
13 (Sigma Aldrich, St.Louis, MO, USA). Analysis on agarose gels and spectrophotometric
14 methods were used to determine DNA quality and quantity. The purified DNA solution
15 containing 50 ng/ μ l of DNA was used for all the further analyses. In addition, milk yield
16 records of 432 cows were obtained from the Cattle Breeders' Association of Turkey.
17 According to Akman and Kumlu [34], cows with consecutive lactations and calving
18 intervals of 300-650 days were taken into account for the variance analysis.
19 Additionally, in the records of the cows to be included in the analysis, attention has
20 been paid that the lactation period should not be shorter than 220 days and the milk
21 yield should not be less than 2000 kg [34].

22 Allele discriminations were based on size differentiation of κ -casein, β -lactoglobulin,
23 prolactin and DGAT1 genetic markers. Identification of these genotypes was performed

1 by the PCR-RFLP method. Extracted DNA samples were amplified by PCR technique,
2 using the primers are given in Table 1. PCR amplifications were prepared in a 50 μ l
3 volume containing 0.2 μ M of each primer, 4 μ l 10X buffer, 25 mM MgCl₂ , 10 mM
4 dNTPs, 1 U Taq DNA polymerase and 100 ng template DNA. PCR reaction steps were
5 optimized for each locus considering the annealing temperatures of the used primers.
6 Amplified PCR products were digested with specific restriction enzymes (Table 1) and
7 then these digested products were separated by electrophoresis on 3% agarose gel and
8 were visualized under ultraviolet light after staining with ethidium bromide.

9 Descriptive and dispersion of allele frequencies were performed by direct counting from
10 the genotype identified in the photo documentation of electrophoresis of the PCR-
11 RFLP. Direct counting was used to estimate phenotype and allele frequencies of β -
12 lactoglobulin, κ -casein, prolactin and DGAT1 genetic variants [35]. The probability of
13 Hardy-Weinberg equilibrium associated with the observed genotypic frequencies was
14 calculated using the chi-square (χ^2) test for the population. The POPGENE [36] package
15 program was used to determine the observed and expected heterozygosity values. After
16 standardizing the data, the relationships between genotypes and milk yield were
17 analyzed using the SAS statistical package program [37] according to the model below.

$$18 \quad Y_{ijkl} = \mu + a_i + b_j + c_k + \beta \text{age}_{ijkl} + e_{ijkl},$$

19 Where, Y_{ijkl} represent the record of 305 days milk yield, μ is overall the mean, a_i is
20 effect of the i -th farm, b_j is effect of the j -th lactation number, c_k is effect of the k -th
21 genotype, β is the regression coefficient, age_{ijkl} is effect of age, e_{ijkl} is random residual
22 effects.

23 **3. Results**

1 Target fragments of cattle κ -casein, β -lactoglobulin, prolactin and DGAT1 genes were
2 amplified from the genomic DNA of 517 Holstein cows registered to the Cattle
3 Breeders' Association of Turkey in Antalya province. After the PCR products obtained
4 for each locus were treated with the appropriate RE enzymes, target RFLP fragments
5 were visualized under ultraviolet light (Figure 1).

6 In the present cows, the κ -casein gene was amplified as a 350 bp fragment comprising a
7 part of exon V of the genomic DNA. Digestion of the PCR products with *HinfI* enzyme
8 revealed three genotypes as AA (134/132 and 84 bp), AB (266, 134/132 and 84 bp) and
9 BB (266 and 84 bp) (Picture 1a). In the β -lactoglobulin gene located in exon IV,
10 digestion of the 247 bp PCR products with *HaeIII* enzyme revealed three genotypes as
11 AA (148 and 99 bp), AB (148, 99 and 74 bp) and BB (99 and 74 bp) (Picture 1b). The
12 prolactin gene located in exon V, digestion of the 156 bp PCR products with *RsaI*
13 enzyme revealed three genotypes as AA (156 bp), AB (156 and 82/74 bp) and BB
14 (82/74 bp) (Picture 1c). Digestion of the 411 bp PCR products with *CfrI* enzyme
15 revealed three genotypes as AA (208/205 bp), AK (411 and 208/205 bp) and KK (411
16 bp) for the DGAT1 gene located in exon VIII (Picture 1d). It is noteworthy that an
17 unexpected fragment (411 bp) was observed for AA genotype in the DGAT1/*CfrI*
18 polymorphism. This could be attributed to different reasons such as higher volume of
19 PCR products and/or lower volume of restriction enzyme.

20 Gene and genotype frequencies were calculated using the gene counting method of Nei
21 [35] by taking into consideration the genotypes obtained after the PCR-RFLP method
22 (Table 2). The calculated chi-square (χ^2) values showed that the current population was
23 in Hardy-Weinberg equilibrium for all loci.

1 Although a total of 517 cows were genotyped for κ -casein-*HinfI*, β -lactoglobulin-
2 *HaeIII*, prolactin-*RsaI* and DGAT1-*Cfr1* polymorphisms, milk yield records of only 432
3 cows were accessible. Therefore, both genotype and milk yield data of 432 cows were
4 utilized for association analysis using SAS MIXED procedure (37). Table 3 presents the
5 mean milk yields according to genotypes determine obtained from Holstein cows for
6 305 days of milking. While the highest milk yield was found in cows with homozygous
7 BB genotype in the prolactin gene region, the lowest milk yield was detected in cows
8 with homozygous BB genotype in the κ -casein gene region. (Table 3).

9 The results of association analysis showed that the genotype differences of κ -casein, β -
10 lactoglobulin and DGAT1 loci had no effect on milk yield, whereas *RsaI* polymorphism
11 of the prolactin gene has a significant effect on milk yield ($p < 0.05$) (Table 4). In
12 addition to the results above, it was seen that the differences between farms and
13 lactations and lactation x farm and age x lactation interactions in terms of milk yield in
14 existing all loci were significant ($P < 0.05$) (Table 4).

15 According to analysis via the POPGENE software package program, it can be said that
16 the differences between the observed and expected genotype frequency values are not
17 significant in the studied population (Table 5). It is seen that the heterozygosity
18 observed in all loci in the current population in Hardy-Weinberg equilibrium is higher
19 than the expected heterozygosity. This situation can be explained by the use of bulls
20 with different genotypes in artificial insemination for the current population.

21 **4. Discussion**

22 When κ -casein gene *HinfI* polymorphism is examined within the scope of the study, it is
23 seen that the A allele (0.8279) is quite common compared to the B allele (0.1721) in the
24 current population. Similar results were reported in previous studies conducted on

1 Holstein-Friesian and its crossbreds [1-10,13]. Similarly, in most studies performed on
2 native (Turkish gray, East Anatolian Red, South Anatolian Red, Anatolian Black) and
3 other European (Brown Swiss) cattle breeds raised in Turkey, the A allele frequency of
4 κ -casein gene has been reported to be high [6,8,10,12]. On the other hand, Akyüz et al.
5 [8] reported that the A and B allele frequencies are close to each other for Anatolian
6 Black and Brown Swiss cattle breeds.

7 The κ -casein B allele was found to be one of the most well-known alleles related to
8 cheese quality [1]. Previous studies reported that cows with the AA genotype have
9 higher milk yield [4,9], but they have a negative relationship with protein yield and
10 protein percentage [2,4]. On the other hand, Hristov et al. [11] found that cows with the
11 BB genotype have a 15% higher milk yield than cows with the AB genotype. It has
12 been reported that the κ -casein gene B variant is associated with the increase in milk
13 protein and fat content, which are important in cheese production [2,9]. However,
14 Bartonova et al. [7] put forward that there is no effects of the *HinfI* polymorphism of the
15 κ -casein gene on the fat and protein yield.

16 In our study, the effect of the κ -casein gene polymorphism on milk yield is not
17 statistically significant. Similar results were also found in some studies performed on
18 breeds in Turkey [6]. The A allele of κ -casein gene was found to be associated with
19 high milk yield and low protein content, while the B allele was associated with high
20 protein content and milk quality, on the other hand, low milk yield [1,2,4,9,12]. In the
21 light of previous studies, it is thought that it will be useful to consider the positive effect
22 of the κ -casein gene, especially the B allele, on cheese production for selection studies.

23 In the present population, the B allele frequency (0.5338) was relatively higher than the
24 A allele frequency (0.4662), while the AA and BB genotype frequencies were almost

1 the same distribution for the β -lactoglobulin/*HaeIII* polymorphism. These results were
2 similar to many studies conducted especially on Holstein Friesian and its crossbreds
3 [4,6,9,10,16,17]. Some other studies on Holstein cattle, the β -lactoglobulin B allele was
4 found quite higher compared to the A allele [3,5]. Different results were found in
5 studies investigated on other cattle breeds raised in Turkey. The A and B allele
6 frequencies were found to be close to each other in Brown Swiss and Gray breeds [18].
7 While the B allele was found to be higher in South Anatolian Red [10,18], Brown Swiss
8 [17], Anatolian Black [10] and East Anatolian Red [10] breeds, the A allele was
9 detected at higher frequency in Simmental cattle [6].

10 Similar to our study, it was found that the genotypes obtained by the *HaeIII*
11 polymorphism of the β -lactoglobulin gene had no effect on milk yield in the studies
12 conducted by Ahmadi et al. [4] and Mohammadi et al [9]. In addition, it was reported
13 that the effect of genotype differences of the β -lactoglobulin gene on fat content was not
14 significant. It was reported that the protein content in the milk of animals with the BB
15 genotype in terms of the β -lactoglobulin gene was higher than that of animals with other
16 genotypes in Holstein cattle [4,9]. At the same time, Karimi et al. [15] reported that the
17 difference in milk protein content among genotypes of the β -lactoglobulin gene was not
18 statistically significant. Additionally, it was reported that the effect of the β -
19 lactoglobulin AB and AA genotype had an effect on milk yield, but this effect was not
20 statistically significant [15].

21 The present study showed that in terms of the prolactin/*RsaI* polymorphism, the A allele
22 (0.8762) is quite common in Holstein cows compared to the B allele (0.1238). This
23 result was similar to previous studies on Holstein and its crossbreds [8,19-26].
24 Similarly, the A allele was detected at high frequencies in cattle breed raised in Turkey

1 (0.6617-0.8610), except for Anatolian Black (A and B frequencies are close to each
2 other) [8,21,38].

3 After analyzes made by the SAS program, it was determined that the effect of the *RsaI*
4 polymorphism of the prolactin gene on milk yield was significant ($p < 0.05$). In
5 particular, it was detected that the milk yield of animals with the B allele was higher
6 (Table 4). However, it should not be overlooked that animals with the BB genotype are
7 very few. It is thought that it will be useful to evaluate this information obtained in
8 selection studies in dairy cattle breeding. Similarly, in some studies, it has been
9 determined that the BB genotype has a significant effect on milk yield or has a tendency
10 to affect it positively [22,26]. On the other hand, Khatami et al. [20] and Kepenek [21]
11 reported that the BB genotype formed by the *RsaI* polymorphism of the prolactin gene
12 has a negative relationship with milk yield, but this relationship is not statistically
13 significant.

14 In some studies, it has been stated that the milk yield of Holstein cows with the AA
15 genotype is higher than that of cows with other genotypes [21,23,26]. In other studies, it
16 has been reported that the milk yield of Holstein cows with the AB genotype is higher
17 than the milk yield of cows with other genotypes [20,25]. However, there are also
18 studies reporting that the *RsaI* polymorphism of the prolactin gene has no statistically
19 significant effect on milk yield [19,20,24].

20 The significant effect of the prolactin/*RsaI* polymorphism on milk fat content was
21 reported in previous studies [19,25]. Dybus et al. [19] found that the effect of the
22 prolactin gene on fat yield and fat percentage in milk was lower in animals with the AA
23 genotype than animals with the AB and BB genotype, and this difference was
24 statistically significant. Alipanah et al. [22] found that the milk of animals with the BB

1 genotype had a higher fat yield compared to the others (BB> AA> AB), and the milk of
2 animals with the AB genotype was higher in terms of fat content, but these results were
3 not statistically significant. However, in some studies, it has been reported that the milk
4 of animals with the AA genotype has higher fat content [20,25].

5 Dybus et al. [19] conclude that the effect of the prolactin gene *RsaI* polymorphism on
6 the protein content of milk is important, on the other hand, Alipanah et al. [22] argue
7 that the effect of these genotypic differences is not statistically significant. Singh et al.
8 [26] reported that the prolactin gene was associated with the number of somatic cells.
9 They reported that the somatic cell count in milk of cows with the AA and AB
10 genotypes was lower than those with the BB genotype, depending on this result, cows
11 with the BB genotype would have higher resistance to mastitis [26].

12 As a result of the *CfrI* polymorphism of the DGAT1 gene, 3 genotypes (KK, KA and
13 AA) could be determined in this study where the K allele (0.6441), known as the lysine
14 variant, was quite common in Holstein cattle breed compared to the A allele (0.3559)
15 known as the alanine variant. This result was similar to some studies performed on
16 Holstein Friesian breed [28,30]. On the other hand, there are many studies in which the
17 A allele is quite high compared to the K allele for this breed [27,32,33,39]. In other
18 studies, it has been reported that the K and A allele frequencies are close to each other
19 in Holstein cattle breed [21,23]. After analyzes made by the SAS program, any effect of
20 genotype differences of the DGAT1 gen on milk yield could not be determined ($P >$
21 0.05).

22 According to the study of Bal and Akyüz [39], A allele was detected at higher
23 frequencies in Anatolian Black. On the other hand, the K allele was higher frequencies
24 (0.7000-0.9250) in other native cattle [21,38,39].

1 In previous studies, it was concluded that the milk of animals with the K allele encoding
2 lysine has a particularly high fat yield, but low protein and milk yield [21,28,30-32]. In
3 addition, while the effects of the K allele on milk yield characteristics were found to be
4 statistically significant, Nowacka-Woszuk et al. [30] and Bobbo et al. [33] reported that
5 the effect of the K allele on fat yield and Spelman et al. [28] on protein yield was not
6 statistically significant. It was reported that the A allele had a significant effect on milk
7 and protein yield, while the fat content was low [27,29,30,32].

8 **Conclusion**

9 It is thought that polymorphisms in κ -casein, β -lactoglobulin, prolactin and DGAT1
10 genes can be used as markers to improve milk yield characteristics in cattle breeds.
11 Also, it was determined that the effect of the prolactin gene BB genotype on milk yield
12 was significant. This result can be used as a selection criterion and it would also be
13 beneficial to increase the number of studies investigating the effects of milk protein
14 gene polymorphism on milk yield characteristics. In this context, it will be able to make
15 significant contributions to milk processing technology by pre-determining of genetic
16 structure of cows and candidate bulls to be used in breeding in terms of the gene regions
17 in said and accordingly selecting them.

18 **Acknowledgement**

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8 **Table 1.** The primer sequences and restriction enzyme of each locus

Gene	Sequences 5'-3'	Enzyme	Reference
κ-casein	F: ATCATTTATGGCCATTCCACCAAAG R: GCCCATTTCGCCTTCTCTGTAACAGA	<i>HinfI</i>	Patel et al. (4)
β-lactoglobulin	F: TGTGCTGGACACCGACTACAAAAA R: GCTCCCGGTATATGACCACCCTCT	<i>HaeIII</i>	Patel et al. (4)
Prolactin	F: CGGAAGTCCTTATGAGCTTGATTCTT R: GCCTTCCAGAAGTCGTTTGTTTTC	<i>RsaI</i>	Dybus et al. (20)
DGAT1	F: GCACCATCCTCTTCCTCAAG R: GGAAGCGCTTTCGGATG	<i>CfrI</i>	Kaupe et al. (30)

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Table 2. Genotype and allele frequencies for studied loci

Locus	Genotype frequencies					Allele frequencies			$(\chi^2)^*$
	AA	AB	BB	AK	KK	A	B	K	
κ -casein	0.6925	0.2708	0.0367	-	-	0.8279	0.1721	-	1.2875 ^{ns}
β -lactoglobulin	0.2302	0.4720	0.2978	-	-	0.4662	0.5338	-	1.3857 ^{ns}
Prolactin	0.7621	0.2282	0.0097	-	-	0.8762	0.1238	-	1.4022 ^{ns}
DGAT1	0.1374	-	-	0.4371	0.4255	0.3559	-	0.6441	1.1174 ^{ns}

* Chi-square values, ns. Not significant $P > 0.005$

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8 **Table 3.** Milk yield obtained from Holstein cows for 305 days of milking according to
9 loci and genotypes

		Locus											
		κ-casein			β-lactoglobulin			Prolactin			DGAT1		
		MY	N	SD	MY	N	SD	MY	N	SD	MY	N	SD
Genotypes	AA	6296	303	52.56	6223	106	91.16	6261	333	50.35	-	-	-
	AB	6239	113	103.84	6323	202	66.21	6282	96	107.32	-	-	-
	BB	6111	16	180.82	6242	124	90.25	8311	3	1270.71	-	-	-
	KK	-	-	-	-	-	-	-	-	-	6318	188	73.54
	KA	-	-	-	-	-	-	-	-	-	6218	203	62.74
	AA	-	-	-	-	-	-	-	-	-	6376	41	169.90
	AA	-	-	-	-	-	-	-	-	-	6376	41	169.90

10 MY: Milk yield (kg), N: Number of animals (head), SD: Standard deviation

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Table.4 Variance analysis results of loci

		κ-casein	β-lactoglobulin	Prolactin	DGAT1
Fixed Effects	DF	Pr > F	Pr > F	Pr > F	Pr > F
Age	1	0.1939	0.1837	0.2484	0.1999
Lactation	2	0.0047*	0.0058*	0.0012*	0.0084*
Farm	5	<.0001*	<.0001*	<.0001*	<.0001*
Genotype	2	0.6539	0.5341	0.0311*	0.9139
Lactation x Farm	9	0.0006*	0.0011*	0.0020*	0.0005*
Age x Lactation	2	0.0196*	0.0207*	0.0387*	0.0349*
Lactation x Genotype	4	0.9862	0.5803	0.2496	0.5436

* Significant, p < 0.05, DF. Degree of freedom.

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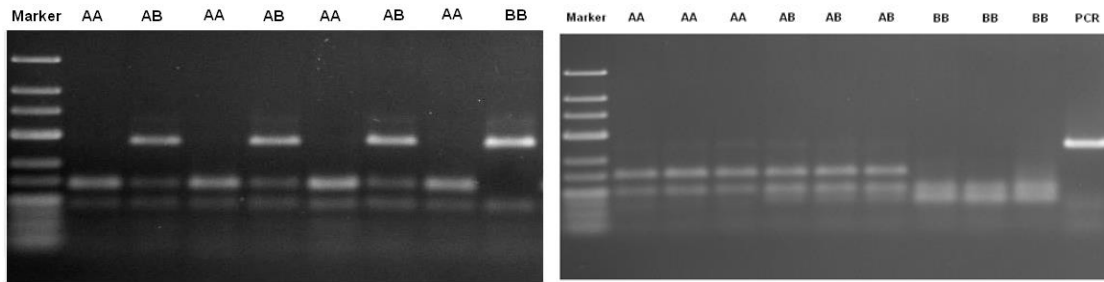
8 **Table 5.** Results of heterozygosity and homozygosity statistics of loci

Loci	N	Observed Heterozygosity (Ho)	Observed Homozygosity	Expected Homozygosity	Expected Heterozygosity (He)	Mean He
κ-casein	517	0.5280	0.4720	0.5018	0.4982	0.4977
β-lactoglobulin	517	0.7292	0.2708	0.7147	0.2853	0.2850
Prolactin	517	0.7718	0.2282	0.7829	0.2171	0.2169
DGAT1	517	0.5416	0.4584	0.5657	0.4343	0.4407
Mean	517	0.6475	0.3525	0.6396	0.3604	0.3601
Standard Deviation		0.1209	0.1209	0.1312	0.1312	0.1311

9 N: Number of animals (head)

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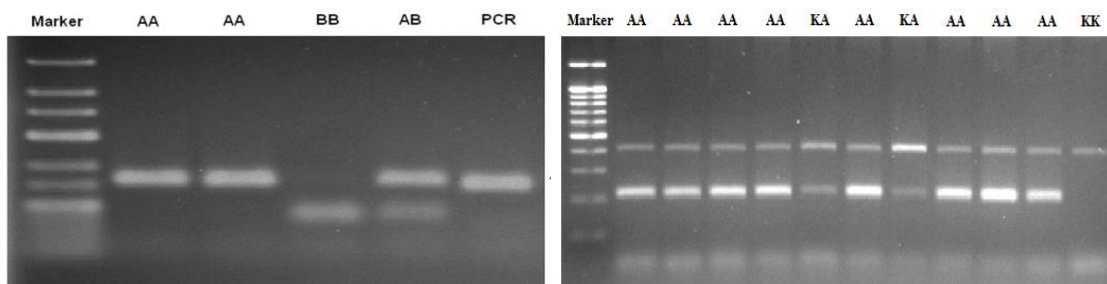
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(a)

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(d)

11 **Figure 1.** Agarose image of RFLP analysis (a) *HinfI* polymorphism of κ -casein gene, AA genotype;
12 134/132:84 bp, BB genotype; 266:84 bp, AB genotype; 266:134/132:84 bp. (b) *HaeIII* polymorphism of
13 β -lactoglobulin gene, AA genotype; 148:99 bp, BB genotype; 99:74 bp, AB genotype; 148:99:74 bp. (c)
14 *RsaI* polymorphism of prolactin gene, AA genotype; 156 bp, BB genotype; 82:74 bp, AB genotype;
15 156:82:74 bp. (d) *CfrI* polymorphism of DGAT1 gene, AA genotype; 208/205 bp, KK genotype; 411 bp.
16 AK genotype; 411: 208/205 bp.

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