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DETERMINATION OF BETALACTOGLOBULIN GENE POLYMORPHISM IN KIVIRCIK SHEEP

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Abstract: This study was carried out to determine the genetic polymorphism of the β-lactoglobulin (β-Lg) gene for indigenous Kivircik breed in Turkey. As to find the Kivircik Sheep is limited as purebred, this breed was raised and maintained in special farm with small numbers as pure breeds. Due to animal material was constituted 48 heads as purebred Kivircik sheep. The study was realized by means of PCR-RFLP methods. Also, two genetic alleles (A and B) and three genotypes (AA, AB and BB) were detected for this purebred. The gene frequencies of β-Lg A and B were calculated as 0.469 and 0.531 in Kivircik sheep. The population was found in equilibrium according to Hardy-Weinberg. At the same time, Some F statistics values were calculated like effective alleles number (Ne), observed heterozygosity (Ho), expected heterozygosity (He) and fixation index (Fis); 1.992, 0.396, 0.498 and 0.205, respectively.

Keywords: Kivircik, β-Lg, Milk protein, Polymorphism, PCR-RFLP

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1. Inrtoduction

2873 Determination of genetic structure in farm animals is very important from the point of animal breeding and raising. Benefiting from polymorphomism presented by animals' type of milk protein, it is aimed that to reveal animals' genetic structure. Factors indicating polymorfic feature are characteristics that determined by several gene pair. Pioneer researches relevant to those factors were realized by Ascaffenburg, Drewry and Ashton, in 1957 (Aschaffenburg and Drewry, 1957; Ashton, 1957). In many of the studies about polymorphism of milk protein, significant relationships between yield features and genotypes were found and determination of milk proteins' genotypes as a selection criterion were suggested. (Mclean et al., 1984; Rensburget al., 1990; Ng-Kwai-Hang et al ,1986; Khaertdinov, 1990; Ng-Kwai- Hang et al ,1990).

Milk protein includes the component which has several different and featured protein combination. Especially complex of casein is known as main fraction of milk proteins. Casein is easily separating by precipitation with acid. The rest of the proteins are whey protein or serum proteins. Whey protein dissolving in semi-saturated ammonium solution is named alpha-lactoalbumin and if it is not dissolving in semi-

saturated ammonium then it is named beta-lactoglobulin (β-lg). Caseins, beta-lactoglobulin and alpha-lactalbumin are syntesized in the mammary epithelial cells. Contary to this, immunoglobulin and serum albumin are absorbed from the blood. (Demirci, 1995).

In several researches, it was reported that locus of β-Lg is polymorphic and possibly, between the alleles, there could be difference in yield and milk processes. (Anton et al., 1999; Baranyi et al., 2010) Anton et al. (1999) were found that in four different dairy sheep breeds, gene frequencies of β-Lg A alleles are 0.3478, 0.6857, 0.5650 and 0.4730, respectively. Mroczkowski et al. (2004) were reported that in Polish Merinos Sheep, β-Lg BB genotypes' protein content is higher than β-Lg AA ve AB types. In Hungarian Merinos and English Dairy Sheep, Anton et al. (2005) found the highest milk yield and milk contents in β-Lg AB genotypes. Celik and Ozdemir, (2006), were found that the milk obtained from β-Lg BB genotypes in Ivesi and Morkaraman Sheep is advantageous for cheese production. Elmaci et al. (2006), were found allele gene frequencies of β-Lg A and β-Lg B in 29 heads of Kivircik, 38 heads of Gokceada, 41 heads of Sakiz Sheep breeds as 0.7759-0.2244; 0.9756-0.0244 and 0.7632-0.2368, respectively. Michalova and Krupuva (2009), were found gene frequencies of β-Lg A and β-Lg B in Cigaja and Valachian Slovakian native sheep breeds, as 0.52-0.48 and 0.60-0.40, respectively. Furthermore, there was no determination between two breeds from the point of milk yield and lactose, but they found significant differences from the point of β-Lg genotypes ($P<0.05$). As to Chinese Chopper Sheep allele gene frequencies of β-Lg A and β-Lg B were found 0.3047 and 0.6953, respectively and significant differences were found between β-Lg (AA, AB and BB) genotypes from the point of lactation period and lactation milk yield ($P<0.05$) (Erdogan, 2009). Araro et al. (2010) researched polymorphism of β-Lg in 15 different sheep breeds breeding in India. They determined two allele genes as A and B in sheep, and in these genes, they determined AA, AB and BB genotypes. They found gene frequencies of β-Lg at the highest frequency for overall breeds. Baranyi, et al. (2010), were found allele gene frequencies of β-Lg A and β-Lg B in Ivesi and Racka Sheep breeding in Hungary, as 0.4796-0.5204 and 0.5764-0.4236, respectively. Elmaci et al. (2012), reported allele gene frequencies of β-Lg A and β-Lg B in 29 heads of Karacabey Merinos Sheep breed as 0.7791-0.2209. In researches done by several researchers, because there were significant relations between types of milk protein with milk yield and composition features, some genotypes were presenting selective advantage, and for this reason it was offered to use these genotypes as mark assisted selection. Therefore, it is important to know existing genetic structure in terms of Kivircik sheep's milk serum protein which was also known as β-Lg locus.

2. Material and Methods

2.1. Animal Material

Animal material of study were obtained from 48 Heads Purebred Kivircik sheep which were under protection, in Kirklareli province. This sheep breed is one of the thin-tailed native sheep breed of Turkey. Especially meat of Kivircik sheep is preferred widely by consumer. Nonetheless, in recent years, with pressure of crossbreeding, it is getting hard to find them purely. For all that, this breed is raised as crossbreed in Marmara Region of Turkey.

2.2. DNA Isolation

DNA was isolated from the neck veins (Vena jugularis) of the animals by 10 ml blood EDTA tubes. Genomic DNA was extracted from total blood samples using fenol/kloroform method (Sambrook et al. 1989). The purity and quantity of the DNAs obtained were determined spectrophotometrically by using nanodrop.

2.3. PCR Reaction and RFLP Application

Beta-lactoglobulin (β-Lg) is one of the milk proteins that is polymorphic in sheep and the gene encoding the protein is located on ovine chromosome 3. Variants of β-Lg loci was identified by PCR-RFLP technique on genomic DNA (Feligini et al. 1998 and Anton et al. 1999b). In the first step, the 120 bp long PCR products in the exon 2 region of the β-Lg gene were digested by the restriction enzyme of Rsa1, and the two alleles were identified as β-Lg A and β-Lg B. In the second step, PCR products with size of 105 bp in the exon 5 region of the β-Lg gene were cut by Msp1 as the restriction enzyme, and the two alleles were determined as β-Lg A and β-Lg C.

PCR reaction was carried out using 50-75 ng of the genomic DNA isolated from the blood samples, 100-150 nM from each forward and reverse primers as 5'-CAACTCAAGGTCCCTCTCCA-3' and 5'-CTTCAGCTCCTCCACGTACA-3', respectively, 200 µM from each dNTP, 2.5 µL from 1X PCR buffer solution, 1.5 mM MgCl2, and 1.25 U of Taq DNA Polymerase in 30 µl reaction volume.First, in order to identify β-Lg A and β-Lg B varyants, genomic DNA samples were amplified using T100 Thermal Cycler (Bio-Rad Laboratories, Inc., USA) with the following conditions: an initial denaturation step at 95°C for 5 min followed by 35 cycles of denaturation for 95°C for 45 sec, an annealing for 60°C for 45 sec, an extension for 72°C for 45 sec and a final extension 72°C for 7 min.

The presence of the β-Lg C allele, a subtype of the β-Lg A allele, was then investigated using 105 bp sheep beta-lactoglobulin fragment as a PCR product.PCR reaction, was conducted in 30 µL reaction volume containing 100-150 nM of each primer, 200 µM of each dNTP, 1.5

mM MgCl2, 2.5 µL of 1x PCR buffer and 1.25 U of Taq DNA Polymerase. The forward and reverse primers were 5'-TCAGGACCCCGCGAGGTGGACAAC-3 'and 5'-CCTCCAGCTGGGTCGGGTTGAAG-3',respectively.PCR reaction was performed at 94°C for 1 minute for initial denaturation followed by 30 cycles of 94°C for 15 sec of denaturation, 60°C for 1 minute of an amplification, 72°C for 10 sec of extension and 72°C for 10 min of final extension processes.

The obtained PCR products were run for 45-60 minutes at 90-100 V to observe if the desired region were amplified by 2% agarose gel electrophoresis containing ethidium bromide. Then 10 µL of the PCR product was digested at 37°C for 3 hours with 8 U RsaI and 10 U MspI restriction endonuclease enzymes in total volume of 20 µL reaction, respectively. After digesting PCR product, the β-Lg variants were determined relative to the bands occurring on the gel under UV light after the DNA fragments were run on a 3% agarose gel.

2.4. Statistical Analysis

Allele gene frequencies of genotypes were calculated according to direct gene counting method. As to Hardy-Weinberg, if the population is at genetic equilibrum or not were tested with chi square analysis. Furthermore, the F statistic values were calculated to show the genetic structure and diversity of the population. Using PopGene32 programme (Yeh et al. 2000). These values are given as effective alleles number (Ne), observed heterozygosity (Ho), expected heterozygosity (He) and fixation index (Fis).

The proportion of homozygosity occurring in genotypes at a given locus as a result of inbreeding is the Fis value. This value is calculated as Fis = (He-Ho) / He. If this value is also positive value, it indicates the lack of heterozygous individuals in population (Nei, 1973).

3. Results and Discussion

In the research, genotyping by β-Lg locus was made on purebred Kivircik sheep. As a result of genotyping made according to bands obtained after enzymedigesting with RsaI and MspI, while 13 β-Lg AA, 19 β-Lg AB and 16 β-Lg BB genotypes were seen in Table 1.There was nochanceseen on β-Lg CC genotype. As to related allele gene frequencies found for β-Lg A and β-Lg B alleles as 0.469 and 0.531, respectively. In the genetic analysis of population, according to Hardy-Weinberg law, it is reported that the population was at genetic equilibrium as a result of chi square analysis (P>0.05). Otherwise, according to some calculated F statistics it is calculated that the effective allele number (Ne) as 1.992, observed heterozygosity (Ho) as 0.396, expected heterozygosity (He) as 0.498 and fixation index Fis as 0.205. Approaching of fixationindex to zero were defining as the herd was in genetic equilibrium and increasinghomozigozysity, in other words, coefficient of inbreeding resulting from inbreeding for a definite locus. Agarose gel electrophoresis band patterns after digestion with RsaI and MspI endonucleases within related regions of the sheep β-Lg gene were given in Fig. 1 and 2.

Table 1. The Gene and Genotype Frequencies of β-Lg and F statistic values in Kivircik Sheep

	β-Lg Genotypes			β-Lg Gene Frequences		Genetic Equilibrium Test
	AA	AB	BB	A	B	χ^2
Observed	13	19	16	0.469	0.531	2.09
% Frequency	27	40	33			(p>0.05)
Expected	11	24	13			
Ne	Ho	He	Fis			
1.992	0.396	0.498	0.205			

Ho: Observed Heterozygosity, He: Expected Heterozygosity Fis: Fixation Index Ne: effective alleles number

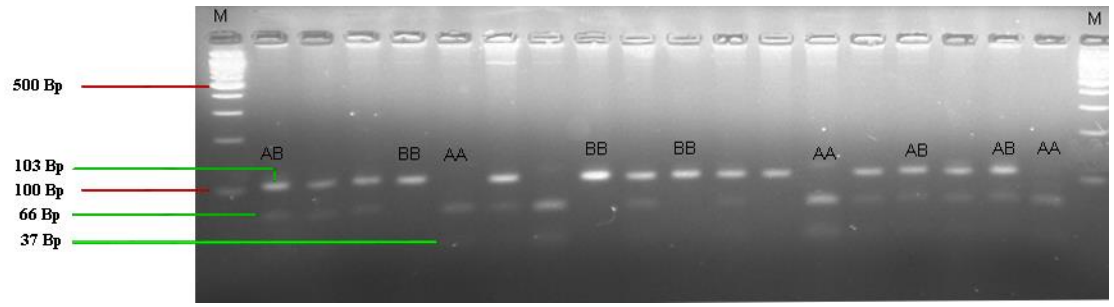

Figure 1. Agarose gel electrophoresis band patterns after digestion with RsaI endonuclease within the exon 2 region of the sheep β-Lg gene

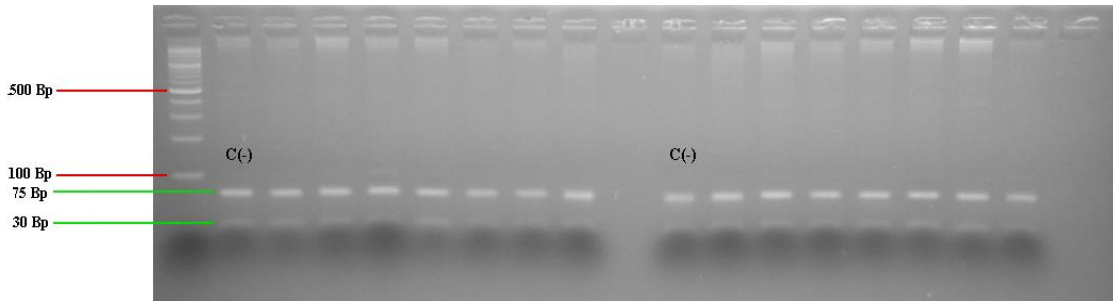

Figure 2. Agarose gel electrophoresis band patterns after digestion with MspI endonuclease within the exon 5 region of the sheep β-Lg gene.

In this research, the β-Lg A gene frequency found as 0.469 in purebreed kivircik breed and in another research this frequencies were found in Kivircik, Gokceada and Sakiz sheep as 0.7759, 0.9756 and 0.7632, respectively (Elmaci et al. 2006). As to other dairy sheep (Ivesi, Dairy English, Tsigaia and Lacaune), they were found as 0.3478, 0.6857, 0.5650 and 0.4730, respectively (Anton et al. 1999). In this study the β-Lg B allele gene frequence was found higher than the β-Lg A. Similarly as to sheep breeds breeding in India, the β-Lg B gene frequencies was found higher frequency for all breeds (Araro et al. 2010).

As to Cine Capari sheep, β-Lg A frequency is found as 0.3047 and from the point of β-Lg genotypes, significant relations were found in terms of lactation period and lactation milk yield ($P<0.05$) (Erdogan, 2009). In Ivesi and Morkaraman sheep, it is reported that milks obtained by β-Lg BB genotypes are much more suitable for cheese production (Celik and Ozdemir 2006). In Karacabey Merinos sheep β-Lg A allele gene frequency was found as 0.7791 (Elmaci et al 2012). As to Ivesi and Racka sheep breeding in Hungary, the β-Lg A allele gene frequency were found as 0.4796 and 0.5764, respectively (Baranyi et al. 2010). While the β-Lg A and β-Lg B allele were seen in all the presented studies, since all the variants C (-), the β-Lg C allele could not been observed in this study.

In this study, the relation between β-Lg and yield Production were not investigated because of data record is not available. But some of the researchers were investigated on it and found significant results. It is reported that in Polish Merinos sheep β-Lg BB genotype milks are higher than β-Lg AA and AB types, from the point of protein content (Mroczkowski et al. 2004). In the Hungarian Merinos and Dairy English sheep, the highest milk yield and milk contents were found in β-Lg AB genotypes (Anton et al. 2005). In the Cigaja and Valachian breeds which are native Slovakian sheep, β-Lg an allele gene frequencies were found respectively 0.52 and 0.60, while there were found no difference between two breeds in terms of milk yield and lactose. There were significant differences were found from the point of β-Lg genotypes ($P<0.05$) (Michalova and Krupuva, 2009).

In the research, firstly, genotyping from the point of β-Lg locus of Kivircik sheep pure breeding in our country, was realized. Furthermore, in the presented researches, the existence of significant relations between the technology of milk production and yield with genotypes. Determination of β-Lg genotypes, is found out that in mark assisted selection. These genotypes can be used as a selection criterion, and as for that the determination of genotypes of animals under our breeding is necessary.

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