Prolactin-RsI\(\text{a}\) gene polymorphism in East Anatolian Red cattle in Turkey

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Abstract
Prolactin (PRL) plays an important role in regulating mammary gland development, secreting milk, and expressing milk protein genes; making it a potential genetic marker and a candidate gene for production traits in dairy animals. The aim of the study was to determine by Polymerase Chain Reaction-Restriction Fragment Length Polymorphism (PCR-RFLP) method the gene and genotype frequencies of PRL gene in native East Anatolian Red (EAR) cattle, which are raised as a genetic resource in Turkey. PCR-RFLP analysis involved the use of the RsAl restriction enzyme. Three patterns of fragments were obtained. The AA, AG, and GG genotype frequencies were 0.07, 0.34, and 0.59 in the cattle population, respectively. For Prolactin-RsAl (PRL-RsAl) polymorphism, the population was in Hardy-Weinberg equilibrium. Heterozygosity was found at a medium rate as 0.338 and the calculated \(F_{IS}\) value was 0.072.

Keywords: genetic resource, mammary gland, prolactin gene, PCR-RFLP

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Introduction
Polymorphic genes involved in the secretion of milk are important as candidate genes, and could be used in indirect selection of livestock because of their relationships with quantitative traits (Micekiene et al., 2006; Alipanah et al., 2008; Alfonso et al., 2012). Prolactin (PRL) plays an important role in regulating mammary gland development, expressing milk protein genes and secreting milk (Brym et al., 2005). Therefore, the PRL gene is potentially a strong genetic marker for the improvement of livestock. The gene consists of 5 exons and 4 introns, and is 10 kb in size, encoding the 199 amino acid in the BTA23 (Camper et al., 1984; Freeman et al., 2000; Dybus, 2002). PRL is secreted from the pituitary gland and cells, mainly the lymphocytes, and has an immunostimulatory effect and promotes autoimmunity (Orbach & Shoefield, 2007).

Genetic polymorphism studies have been carried out on the bovine PRL gene sequences. The most important polymorphism was located and identified by RsI\(\text{a}\) endonuclease using PCR-RFLP (Mitra et al., 1995; Brym et al., 2005). These polymorphic structures have been studied by many researchers, who confirmed statistically significant associations between these polymorphic variants and milk production traits in cattle (Dybus et al., 2005; Brym et al., 2005; He et al., 2006; Alipanah et al., 2008; Mehmannavaz et al., 2009; Rorie et al., 2009; Alfonso et al., 2012; Boleckova et al., 2012; Ishaq et al., 2012; Akyuz et al., 2012; Akyuz & Cinar, 2014; Ozkan Unal et al., 2015). It was suggested that PRL variants could be useful in direct selection programmes for improving milk traits in livestock (He et al., 2006; Alipanah et al., 2008; Rorie et al., 2009; Alfonso et al., 2012; Boleckova et al., 2012; Akyuz et al., 2012). Generally, the results showed that PRL-RsI\(\text{a}^{1}\) allele effect was significant for milk and protein yield, where the PRL-RsI\(\text{a}^{2}\) allele was unfavourable for milk and protein yield, but favourable for fat yield.

Table 1 presents the findings of various researchers, who reported allele frequencies in the PRL-RsI\(\text{a}\) region in buffalo and cattle breeds. The PRL-RsI\(\text{a}^{1}\) polymorphisms have been reported as A/B or A/G in some studies.

The EAR native cattle breed is genetically the most distant compared with other breeds because it is a native breed in the vicinity of the Near East, which is known as the first centre of domestication (Ozdemir & Dogru, 2009; Dogru et al., 2012). The objectives of the present study were to determine PRL-RsI\(\text{a}\) polymorphism, and estimate the gene and genotype frequencies in native EAR cattle in Turkey. Determining the PRL-RsI\(\text{a}\) allele composition particular to the EAR breed would contribute to conservation efforts for this breed because it is raised as a valuable genetic resource.
### Table 1 Polymorphism of PRL-\textit{RsaI} locus in various buffalo and cattle breeds

| References               | Breeds                          | A (\textit{RsaI}) | B (\textit{RsaI}) | References               | Breeds                          | A (\textit{RsaI}) | B (\textit{RsaI}) |
|--------------------------|----------------------------------|--------------------|-------------------|--------------------------|----------------------------------|--------------------|-------------------|
| Mitra \textit{et al.},   | German Black Pied                | 0.80               | 0.20              | Dybus, 2002              | Polish                          | 0.86               | 0.14              |
| 1995                     | Swiss Brown                      | 0.61               | 0.39              |                          | Russian Black                   | 0.71               | 0.29              |
|                          | Sahiwal                          | 0.49               | 0.51              |                          | Russian Red                     | 0.70               | 0.30              |
| Dybus \textit{et al.},   | Black and White                  | 0.85               | 0.15              | Alipanah \textit{et al.},| East Anatolian Red              | 0.56               | 0.44              |
| 2005                     | Jersey                           | 0.31               | 0.69              | 2008                     | South Anatolian Red             | 0.74               | 0.26              |
| Kaplan & Boztepe, 2010   | Brown Swiss                      | 0.82               | 0.18              | Oztabak \textit{et al.}, | Turkish Grey                    | 0.76               | 0.24              |
|                          | Anatolian buffalo                | 1.0                | 0.0               | 2008                     | East Anatolian Red              | 0.70               | 0.30              |
| Sodhi \textit{et al.},   | Hindistan native cattle breeds   | 0.52               | 0.48              |                          | Anatolian Black                 | 0.58               | 0.42              |
| 2011                     |                                  |                    |                   |                          | South Anatolian Red             | 0.76               | 0.24              |
| Verma \textit{et al.},   | Indian Murrah buffalo            | 0.93               | 0.07              | Akyuz \textit{et al.},   | Brown Swiss                     | 0.73               | 0.27              |
| 2012                     |                                  |                    |                   | 2012                     | Holstein                        | 0.86               | 0.14              |
| Alfonso \textit{et al.}, | American Swiss                   | 0.88               | 0.12              |                          | East Anatolian Red              | 0.74               | 0.26              |
| 2012                     | Deoni                            | 0.39               | 0.61              | Akyuz \& Cinar, 2014     | Brown Swiss                     | 0.44               | 0.56              |
| Das \textit{et al.},     |                                  |                    |                   |                          | Zavot                           | 0.65               | 0.35              |
| 2012                     | Nusa Tenggara Barat              | 0.95               | 0.05              |                          | Simmental                       | 0.67               | 0.33              |
| Paramitasari \textit{et al.}, 2015 | South Sulowesi                  | 0.95               | 0.05              |                          | Turkish Grey                    | 0.70               | 0.30              |
|                          |                                  |                    |                   |                          | East Anatolian Red              | 0.68               | 0.32              |
|                          |                                  |                    |                   |                          | Anatolian Black                 | 0.52               | 0.48              |
|                          |                                  |                    |                   |                          | South Anatolian Red             | 0.71               | 0.29              |

### Materials and Methods

Blood samples were collected in a 10 ml vacuum tube containing K3EDTA from the left jugular vein of 71 EAR cattle (17 bulls and 54 cows), which are maintained as a genetic resource in Eastern Anatolia, Turkey. Genomic DNA was extracted from whole blood samples using the Purgene kit (Gentra Systems, Plymouth, Minn., USA) and stored at 4 °C.

For the PRL gene, PRL-\textit{RsaI} Forward:5'-TTC ATG AAG CTG CTC ACC TG-3' and Reverse:5'- TGT GGT TGT TCA GCA TGA AGT-3' primers were designed from the National Centre for Biotechnology Information (NCBI) GenBank sequences (accession nos AB098480 and AF426315) using the Primer3 program (Rozen & Skaletsky, 2000). Amplification reactions were performed in a final volume 20 μl.
containing 1 μM of each primer, 2.5 μl dNTP (D7595) (Sigma, St. Louis, Mo., USA), 0.5 U of Taq DNA polymerase (D1806) (Sigma, St. Louis, Mo, USA), approximately 50–100 ng of template DNA, 5 μl of 10x PCR buffer (catalogue P2192) (Sigma, St. Louis, Mo, USA), 1 μl of 25 mM MgCl₂ and ddH₂O. PCR amplifications were performed in 5 min at 94 °C, 30 cycles of 45 s at 94, 61 and 72 °C, which were followed by final extension at 72 °C for 5 min. The amplified products were digested by using Rsal at 37 °C overnight. To genotype animals for the RFLP, in related region, 8–10 μl PCR reaction mix was used for restriction enzyme digestion, which was performed in 15 μl volume in 0.2 ml sterilized Eppendorf tubes. Each 15 μl digestion mix was electrophoresed in 2.5% agarose gel at 40 V for 2.5 h and DNA was visualized by staining with ethidium bromide under UV light. A standard DNA marker (P1473) (Sigma, St. Louis, Mo., USA) was used.

For each animal, PRL allele frequencies were determined by gene counting. The chi-square (X²) test was used to check whether the population was in H-W equilibrium by GenAlEx 6.5 program (Peakall & Smouse, 2012).

Results and Discussion

PRL gene polymorphisms were investigated by the PCR-RFLP method in native EAR cattle raised as genetic resource in Turkey. The genotyping procedure revealed three patterns of fragments of 210 bp (allele G) and 120 and 90 bp (allele A) for the PRL-Rsal region (Figure 1).

![Figure 1 Polymorphism fragments of the prolactin gene obtained with the enzyme Rsal in agarose gel using 2.5% with ethidium bromide. Lane 1 and 4: genotype GG (210 bp), Lane 2: genotype AA (120 and 90 bp) and Lane 3: genotype AG (10, 120, and 90 bp)](image_url)

Genotype frequencies and allelic frequencies of native EAR cattle are presented in Table 2 and Figure 2, respectively.

**Table 2 Allelic and genotypic frequencies of the Prolactin-Rsal polymorphism, heterozygosity and fixation index and statistical test result for Hardy-Weinberg equilibrium.**

| Genotype | Allele frequency | Heterozygosity and fixation index | H-W X² test |
|----------|-----------------|---------------------------------|-------------|
|          |                 | Ho  | He  | Fis |               |             |
| AA       | 0.07 (5)        |     |     |     | 0.338          | 0.38        |
| AG       | 0.34 (24)       |     |     |     | 0.364          |             |
| GG       | 0.59 (42)       |     |     |     | 0.072          |             |
|          |                 | 0.76| 0.24|     |               |             |


AA, AG, and GG genotype frequencies obtained in PRL-Rsal were 0.07, 0.34 and 0.59, respectively. G allele (PRL-RsalG) frequency was 0.76, and was found to be more prominent than the A allele. This result is similar to the findings of earlier researchers, who reported genotype frequencies in various regions of the world with different breeds and sample sizes (Dybus et al., 2002; Miceikienė et al., 2006; Alipanah et al., 2008; Mehmannahvaz et al., 2009; Rorie et al., 2009; Kaplan & Boztepe 2010; Sodhi et al., 2011; Alfonso et al., 2012; Boleckova et al., 2012; Das et al., 2012; Ishaq et al., 2012). However, Dybus et al. (2005) found a lower G allele frequency in Jerseys.

PRL-Rsal(1) allele (gene) frequency in previous studies on EAR cattle had the greatest frequency and the population was in H-W equilibrium (\(P > 0.05\)) (Oztabak et al., 2008; Akyuz & Cinar, 2012; Ozkan Unal et al., 2015). These results were similar to those of the present study.

Heterozygosity was found at a medium rate as 0.338 for PRL-Rsal polymorphism and the population was in H-W equilibrium (Table 2). Heterozygosity was 0.186 in EAR (Akyuz et al., 2012), 0.038 in Holstein, and 0.33 in Jersey cattle (Brym et al., 2005). Dybus et al. (2005) found 0.28 in Black and White and 0.43 in Jersey. The \(F_{IS}\) value for the region was 0.072 in EAR population. According to this value, despite the small number of the EAR population as a genetic resource, homozygosity was at a low rate.

Molecular genetic techniques have allowed the use of DNA markers associated with various economic traits in promoting efficient selection and breeding strategies of livestock. It is now generally accepted that the PRL gene plays a key role because it regulates mammary gland development, milk protein genes and milk secretion (Alipanah et al., 2008; Othman et al., 2011) Thus, the PRL gene may be a strong candidate gene for economically important production traits. However, in this study, the relationships between PRL-Rsal genotypes and production traits were not established because of lack of records of native EAR cattle.

**Conclusion**

PRL-Rsal polymorphism was investigated by the PCR-RFLP method. It showed the allelic and genotypic frequencies of the PRL-Rsal polymorphism region, heterozygosity and fixation index in the native EAR cattle breed; which are raised as a genetic resource in Turkey. For PRL-Rsal polymorphism, heterozygosity was found at a medium rate and the native EAR cattle population was in H-W equilibrium (\(P > 0.05\)). In this study, the relationships between genotypes and production traits were not established on native EAR cattle. However, it is now generally accepted that PRL plays a key role in the secretion of milk, in regulation of mammary gland development, and the expression of milk protein genes of cattle. Thus, PRL may be a strong candidate gene for economically important production traits. Associations between PRL gene polymorphism and economic traits for the EAR population should be investigated further and evaluated for marker-assisted selection in large numbers of animals, which are required for such studies.

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**Authors’ contributions**

This article was extracted from SZ’s Master of Science thesis. OM wrote the manuscript and was responsible for drafting and submitting it.
Conflict of Interest Declaration

None of the authors of this work has a financial or other relationship with people or organisations that could influence inappropriately or bias the contents of this paper.

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