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Short Communication

A *Prl*/*RsaI* Polymorphism in Exon 3 and 4 of Prolactin Gene in Dairy Cattle

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ABSTRACT

Prolactin is a quantitative trait locus and is a potential genetic marker that can be used in the improvement of production traits in dairy cattle, because prolactin has an important regulatory role in the development of the mammary glands, the secretion of milk and the expression of milk protein genes. It has been seen many studies about *Prl/RsaI* polymorphism found in the exon 3 or exon 4 region of bovine prolactin gene in the literature. The aim of this study was to determine whether the DNA sequence of the *Prl* gene exon 3 or exon 4 in cattle breeds has the *RsaI* polymorphic digestion site. As a result of the study, it has been seen that the *Prl*/*RsaI* specific polymorphic site is on exon 4 in *Bos taurus* cattle breeds, but have been not detected the *RsaI* restriction enzyme digestion site in *Prl* gene exon 3 region reported by many literature and, these results also have been approved by blasted at the NCBI Genbank.

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Prolactin gene, *Prl/RsaI* polymorphic site, Blast, Exon 4, Cattle.

Prolactin is defined as an important lactation hormone due to its regulatory function in the secretion of milk and expression of milk proteins, and the formation and development of mammary glands (Brym *et al*., 2005). Due to these characteristics, it is an important genetic marker that can be used in the breeding of livestock and is used extensively in the studies of genetic polymorphism (Supplementary Table I).

The bovine Prolactin gene (b*Prl*) secreted by the anterior lobe of the pituitary is a polypeptide and composed of five exons and four introns, encodes the 199 amino acid groups of the mature protein found on chromosome 23 (23q21 position) in the bovine genome and is about 10 kb in size (Hallerman *et al*., 1988). The exons of the b*Prl* gene (GenBank accession No: AF426315.1) consist of exon 1: 855 to 936 nt, exon 2: 3661-3842 nt, exon 3: 6186-6293 nt, exon 4: 8321-8500 nt and exon 5: 9129-9388 nt. It has been reported until now that there are more than 20 SNPs on the b*Prl* gene, and most of them have been defined as silent mutations in the intron region (Brym *et al*., 2005; Halabian *et al*., 2008; Uddin *et al*., 2013). From this SNP at the *Prl* gene, G/A-transition creates a restriction site for *RsaI* endonuclease. This polymorphism identified by *RsaI* endonuclease has been investigated by several workers (Lewin *et al*., 1992; Mitra *et al*., 1995; Dybus, 2002; Dybus *et al*., 2005; Brym *et al*., 2005; Alipanah *et al*., 2007; Mehmannavaz *et al*., 2009).

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Firstly, Lewin *et al*. (1992) and Mitra *et al*. (1995) denoted A and B alleles as a result of digestion of 156 bp fragment of *Prl* gene with the *RsaI* restriction enzyme. Three different genotypes were expected: AA in which both the alleles of *Prl* genes were not restricted with the *RsaI* enzyme and only one 156 bp band appeared; AB in which three bands 156, 82 and 74 bp appeared and BB in which only two bands, 74 and 82 bp long appeared. Then, Brym *et al*. (2005) reported that the transition of G into A in position 8398 creates a restriction site for *RsaI* endonuclease on *Prl* gene-exon 4. Digestion of the 294 bp PCR product with the enzyme resulted in two restriction fragments of 162 and 132 bp for AA homozygotes, one uncut fragment of 294 bp for GG homozygotes, and all three fragments for AG heterozygotes.

Many researchers working on the *Prl* gene exon 3 region of different cattle breeds have reported high rate of A allele frequency and low rate of B allele frequency (Lewin *et al*., 1992; Mitra *et al*., 1995; Dybus 2002; Dybus *et al*., 2005; Miceikiene *et al*., 2006; Kepenek, 2007; Alipanah *et al*., 2008; Oztabak *et al*., 2008; Wojdak *et al*., 2008; Ghasemi *et al*., 2009; Rorie *et al*., 2009; Kaplan and Boztepe, 2010; Sharifi *et al*., 2010; Sodhi *et al*., 2011; Akyuz *et al*., 2012; Vikas *et al*., 2012; Verma *et al*., 2012; Alfonso *et al*., 2012; Boleckova *et al*., 2012; Sonmez and Ozdemir, 2015) (Supplementary Table I).

Similarly, many researchers conducting studies on the *Prl* gene exon 4 have reported that the A allele gene has low frequency, but the G allele has high frequency (Brym *et al*., 2005; Mehmannavaz *et al*., 2009; Rorie *et al*., 2009; Dayal Das *et al*., 2012; Schennink *et al*., 2009; Sonmez and

Ozdemir, 2015). Prolactin gene polymorphisms presented in Supplementary Table I, reports allele frequencies in *Prl/RsaI*-exon 3 (called as A/B) or exon 4 (called as A/G) region, in buffalo and bovine breeds. Some researchers who associate the performance characteristics of animals to the identified *Prl/RsaI* polymorphic genotypes consider the regions as being independent of each other, and have reported that the BB genotype in the exon 3 region, and the AA genotype in the exon 4 region are negatively related to the yield (Dybus *et al*., 2005; Brym *et al*., 2005; Kepenek, 2007; Khatami *et al*., 2005; Alipanah *et al*., 2008; Ghasemi *et al*., 2009; Rorie *et al*., 2009; Alfonso *et al*., 2012; Boleckova *et al*., 2012; Ishaq *et al*., 2012).

The aim of this study was to investigate the *RsaI* polymorphism in exon 3 and 4 of *Prl* gene of dairy cattle breeds.

Materials and methods

Blood samples of 50 Holstein cows reared at the Research and Application Farm Faculty of Agriculture, Ataturk University in Turkey were collected from the jugular vein in 10 ml vacuum tube containing K3EDTA. Genomic DNA was extracted from whole blood samples using Purgene kit (Gentra Systems, Plymouth, MN, USA). For *Prl/RsaI* polymorphism, the primers used for exon 3 were (Mitra *et al*., 1995): *Prl*/*RsaI* Forward: 5'-CGA GTC CTT ATG AGC TTG ATT CTT-3', Reverse: 5'- GCCTTCCAGAAGTCGTTTGTTTTC-3', and the primers used for exon 4 were (Brym *et al*., 2005): *Prl/RsaI* Forward: 5'-CAT GGT GAC CTG CAT CCT C-3', Reverse: 5'-ACC CTC ATG CCT CTC ACA TC-3' primers were used. The amplified PCR products of both regions were digested by using *RsaI* at 37°C overnight. To genotype animals for the RFLP, 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. For each animal, Prolactin allele frequencies were calculated by counting the alleles.

The specificity of the above primers revealed by the literature to be specific to the region defined as Prl exon 3 or exon 4 in NCBI Genbank site was investigated by making BLAST.

Results and discussion

The PCR-RFLP results of *Prl/RsaI* polymorphism (*Prl/RsaI* $^{+/-}$) in exon 3 and 4 are shown in Table I.

Brym *et al*. (2005) and Mitra *et al*. (1995) had reported 156 bp band for exon 3 and 294 bp band for exon 4 product, respectively (Table II).

Table I.- The allelic frequencies and genotype numbers of the *Prl*/*RsaI* Polymorphism for both primers pairs.

Prl	Genotype			Allele frequency	
	(*Rsa*$^{+/+}$)	(*Rsa*$^{+/-}$)	(*Rsa*$^{-/-}$)		
	BB	**AB**	**AA**	**B**	**A**
Exon 3	3	19	28	0.25	0.75
	AA	**AG**	**GG**	**A**	**G**
Exon 4	3	19	28	0.25	0.75

Note that *RsaI* enzyme was applied to the same examples.

As a result, while it has been observed that the both primer pairs in the test results indicate the same polymorphism, the same DNA sequence (NCBI, GenBank No: AF426315.1) was obtained when these primers were blasted with nucleotide sequences for specificity checking separately (Fig. 1). The Figure 1 shows exon 4 nucleotide sequences, using primers which had restriction site for *RsaI* enzyme.

The most important polymorphism of the *Prl* gene has been found on exon 4, and this SNP named as *RsaI* polymorphism, no matter which primer pair is used, must be called as A/G polymorphism or *RsaI*$^{+/-}$ in order to avoid misunderstandings (Fig. 1). Moreover, in the bovine *Prl* gene, exon 3 region certainly do not have the *RsaI* restriction enzyme digestion site and *RsaI* polymorphism. Halabian *et al*. (2008) have reported 156 bp fragment by PCR-SSCP method in exon 3, and reported that they had identified 4 SNP. However, it is detected that the DNA sequence of the region examined is similar to exon 4. Furthermore, in the similar studies conducted by different researchers, the exon 3 and exon 4 regions have been shown to produce 156 bp and 294 bp fragment of the *Prl* gene, respectively, but different genotype frequencies have been

Table II.- b*Prl* locus region and fragment size digested by *RsaI* endonuclease.

Region	Primers used for amplification	Fragment size	References
Exon 3	F: 5'-CGAGTCCTTATGAGCTTGATTCTT-3' R: 5'-GCCTTCCAGAAGTCGTTTGTTTTC-3'	AA: 156 nt AB: 156-82-74 nt BB: 82-74 nt	Lewin *et al*. (1992) Mitra *et al*. (1995)
Exon 4	F: 5'-CCAAATCCACTGAATTATGCTT-3' R: 5'-ACAGAAATCACCTCTCTCATTCA-3	GG: 294 nt AA: 162, 132 nt AG: 294, 162, 132 nt	Brym *et al*. (2005)

...CAAAAAATTTTCAATACATATAGGAAAAACCAGGAGTTTTTTAGGTCAATCACTCTGAGCAAAAATCACATGTT
ACCAAATCCACTGAATTATGCTTATTTTAATGAGATTGTTTCTTGTGGTCGTTCAGCATGAAGTCCTTATGAGCT
TGATTCTTGGGTTGCTGCGCTCTTGGAATGACCCTCTGTATCACCTAGTCACCGAGGT^ACGGGGTATGAAAGG
AGCCCCAGATGCTATCCTATCGAGGGCCATAGAGATTGAGGAAGAAAACAAACGACTTCTGGAAGGCATGGAGAT
GATATTTGGCCAGGTGAGCAGCTTCATGAAAGCTTCCTTGCTATTTTCATGAATGAGAGAGGTGATTTCTGTAAT
GAGGAATGAGTTTTGAACTATCTCACTGTACAAGAACACAATTCAGGCCTTCTTTTTCTAGACCGGTGTTACATA
AAGCAAGAACCTGTTCATTCATAGTGATAGATTCTATTGTAAG...

Fig. 1. *Prl* gene exon 4 nucleotide sequences, studied both primer pairs and digested site by the *RsaI* enzyme (for DNA sequence, GenBank: AF426315.1; note that single underlined primer pairs produce 294 bp fragment, and doubled underlined primer pairs produce 156 bp fragment. GTˆAC: *RsaI* restriction enzyme digestion site).

determined (Dayal Das *et al*., 2012; Paramitasari *et al*., 2015; Sonmez and Ozdemir, 2015). Although the both primer pairs indicating the same region have been studied together in these studies, the observation that the gene and genotype frequencies are found to be different can probably be explained as an experimental or sampling error.

Conclusion

In *Bos taurus* cattle breeds shows*RsaI* polymorphism in Prl exon 4 and not in exon 3. It is recommended that similar studies should indicate that the *Prl*/*RsaI* polymorphism is only in the exon 4 region, and the correct way to define these different polymorphisms in the A/G or A/B format should be defined as *Prl*/*RsaI*$^{+/-}$ polymorphism with the presence or absence of the RsaI restriction enzyme recognition site in the *Prl* gene region.

Supplementary material

There is supplementary material associated with this article. Access the material online at: https://dx.doi.org/10.17582/journal.pjz/2020.52.1.sc7

Statement of conflict of interest

The author confirms that he has no conflict of interest with reference to this article.

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Supplementary Material

A *Prl*/*RsaI* Polymorphism in Exon 3 and 4 of Prolactine Gene in Dairy Cattle

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Supplementary Table I.- *Prl*/*RsaI* gene polymorphisms with different breeds in different regions of the world.

References	Breeds	A (*Rsa*⁻)*	B (*Rsa*⁺)	References	Breeds	A (*Rsa*⁻)	B (*Rsa*⁺)
Mitra *et al.* 1995	German Black White	0.80	0.20	Chung *et al.* 1997	Korean cattle breeds	0.68	0.32
	Swiss Brown	0.61	0.39		(cows -bulls)	0.77	0.23
	Sahiwal	0.49	0.51				
Dybus, 2002	Polish	0.86	0.14	Dybus *et al.* 2005	Black and white	0.85	0.15
					Jersey	0.31	0.69
Udina *et al.* 2001	Gorbatov Red	0.91	0.09	Khatami *et al.* 2005	Russian Black White	0.95	0.05
	Ayrshire	0.86	0.14		German Black White	0.61	0.39
	Black Pied	0.80	0.20		Yaroslavl	0.65	0.35
Miceikiene *et al.* 2006	Litvania Dairy cattle	0.87	0.13				
Kepenek, 2007	South Anatolian Red	0.76	0.24	Alipanah *et al.* 2008	Russian Black	0.71	0.29
	East Anatolian Red	0.66	0.34		Russian Red	0.70	0.30
	Anatolian Black	0.56	0.44	Oztabak *et al.* 2008	East Anatolian Red	0.56	0.44
	Turkish Gray	0.70	0.30		South Anatolian Red	0.74	0.26
	Holstein	0.86	0.14				
Kumari *et al.* 2008	Holstein +Jersey	0.77	0.23	Wojdak *et al.* 2008	Holstein-Friesian	0.58	0.42
	Zebu breeds	0.67	0.33				
Ghasemi *et al.* 2009	Montebeliard cow	0.63	0.37	Kaplan and Boztepe, 2010	Brown Swiss	0.82	0.18
					Anatolian Water Buffalo	1.0	-
Sharifi *et al.* 2010	Najdi	0.57	0.43	Sodhi *et al.* 2011	India native cattle breeds	0.52	0.48
Alfonso *et al.* 2012	American Swiss	0.88	0.12	Vikas *et al.* 2012	Frieswal cattle	0.63	.370
Akyuz *et al.* 2012	Turkish Gray	0.76	0.24	Dayal Das *et al.* 2012	Deoni	0.39	0.61
	East Anatolian Red	0.70	0.30	Mahajan *et al.* 2012	Frieswal	0.63	0.37
	Anatolian Black	0.58	0.42	Verma *et al.* 2012	Indian Murrah buffalo	0.93	0.07
	South Anatolian Red	0.76	0.24				
	Brown Swiss	0.73	0.27				
	Holstein	0.86	0.14				
Akyuz *et al.* 2013	Simmental	0.81	0.19	Lazebnaya *et al.* 2013	Yakut	0.73	0.27
	Brown Swiss	0.76	0.24		Yaroslavl	0.65	0.35
	Holstein	0.87	0.13		Bestuzhev	0.68	0.32
					Kastroma	0.75	0.25
Bukhari *et al.* 2013	Frieswal	0.63	0.37	Biradar *et al.* 2014	Murrah Buffalo	1.0	0.0

References	Breeds	A (*Rsa*$^-$)*	B (*Rsa*$^+$)	References	Breeds	A (*Rsa*$^-$)	B (*Rsa*$^+$)
Akyuz ve Cinar, 2014	East Anatolian Red	0.74	0.26	Ozkan Unal *et al.* 2015	Turkish Gray	0.70	0.30
	Brown Swiss	0.44	0.56		East Anatolian Red	0.68	0.32
	Zavot	0.65	0.35		Anatolian Black	0.52	0.48
	Simmental	0.67	0.33		South Anatolian Red	0.71	0.29
Paramitasari *et al.* 2015	Bali	0.95	0.05	Sonmez and Ozdemir, 2015	East Anatolian Red	0.77	0.23
	NTB	0.85	0.15				
	South Sulowasi	0.95	0.05				
References	**Breeds**	**A (*Rsa*$^+$)***	**G (*Rsa*$^-$)**	**References**	**Breeds**	**A (*Rsa*$^+$)**	**G (*Rsa*$^-$)**
Brym *et al.* 2005	Black and White	0.11	0.89	Dayal Das *et al.* 2012	Deoni	0.19	0.81
	Jersey	0.71	0.29				
Mehmannavaz *et al.* 2009	Holstein bulls	0.07	0.93	Khaizaran and Al-Razem, 2014	Freisian	0.71	0.29
					Hybrid	0.94	0.06
					Local breeds	0.82	0.18
Rorie *et al.* 2009	Holstein	0.08	0.92	Paramitasari *et al.* 2015	Bali	0.95	0.05
	Hybrid cattle	0.30	0.70		NTB	0.87	0.13
Boleckova *et al.* 2012	Fleckvieh	0.12	0.88		South Sulowasi	0.94	0.06
Ishaq *et al.* 2012	Nili-Ravi Buffalo	0.00	1	Sonmez and Ozdemir, 2015	East Anatolian Red	0.24	0.76
	Sahiwal	0.19	0.81				
	Achai	0.44	0.56				

*It was called as A/B on the exon 3 and A/G on the exon 4, but the naming in the form of *RsaI* $^{+/-}$ is proposed in the present study.