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Short Communication

ASSOCIATIONS BETWEEN *PRL/RsaI* POLYMORPHISM AND SOME PERFORMANCE TRAITS IN HOLSTEIN CATTLE REARED UNDER ORGANIC CONDITION

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ABSTRACT

The aim of this study was to ascertain the *RsaI* polymorphism of the *Prolactin* (*PRL*) gene of 186 organically reared Holstein cattle by PCR-RFLP method, in order to reveal the genetic variation of the population, and to reveal the relationship between *PRL*/*RsaI* genotypes and some milk yield traits. In the study, the $RsaI^{-/-}$ genotype frequency, $RsaI^{+/+}$ genotype frequency and $RsaI^{-/+}$ genotype frequency of Holstein cattle were determined as 0.26, 0.22 and 0.52, respectively. The $RsaI^{-}$ gene frequency was 0.52, and the $RsaI^{+}$ gene frequency was 0.48. Hardy-Weinberg genetic equilibrium test showed that the distribution of genotype frequencies was determined to be in genetic equilibrium. The relationships between *PRL*/*RsaI* polymorphism and the examined milk yield traits were not significant. As a result, an economic and safe method PCR-RFLP for the detection of genetic markers, was successfully used in the determination of the 3 genotypes ($RsaI^{-/-}$, $RsaI^{-/+}$, and $RsaI^{+/+}$) of Prolactin gene in Holstein cattle reared under organic condition, and the results was similar with previously reported literature.

Keywords: Prolactin, PCR-RFLP, polymorphism, marker, cattle.

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INTRODUCTION

Animal breeding has been carried out by selection based on the prediction of the additive genetic data of animals, which are superior with regards to phenotypic or yield traits. Molecular biology techniques enabled the possibilities to identify the genetic variation in specific loci and to examine the relationship between the variation in QTL (quantitative trait loci) and yield traits. The main goal is to amplify the genetic gain obtained from selection by estimating the genetic value of the animal with higher accuracy. For this purpose today, obligation to use MAS (Marker Assisted Selection) arises. Thus, it has been expressed in investigations that genetic variations on genes may affect quantitative variations in the related phenotype (Tambasco *et al.,* 2003). It has been suggested that the existing polymorphisms within the selected candidate genes can be tested the association with quantitative traits and related polymorphic regions can be used in MAS programs (Wu *et al.,* 2005). Applying the traditional selection method to animals with long generations, such as cattle, sheep's and goats, resulted in slow genetic improvement. Therefore, the speed of selection can be increased by taking advantage of genetic markers that can be determined at an early age regardless of gender bias. Genetic polymorphisms with important functional effects on yield traits have been seen to have the most usage area in association studies.

On Chromosome 23 (Hallerman *et al.,* 1987), the *Prolactin* (*PRL*) is 10 kb in size, consists of 5 exons and 4 introns (Camper *et al.,*1984). It synthesizes a total of 229 amino acids, including 30 signal amino acids and 199 active amino acids (Wolf *et al.,* 1990; Gothard *et al.,* 1996, Cao *et al.,* 2002). The prolactin hormone is mainly secreted by the anterior pituitary of the brain and has multiple functions. The target organ for the prolactin is the mammary gland, which stimulates development and differentiation here (Schradin *et al.,* 2004). Their high levels in the blood, inhibit synthesis and secretion of hypofizar gonadotropins and steroid synthesis in ovaries. In addition to stimulating milk secretion, it accounts for growth, reproduction, osmoregulation immunology, gonadotropin secretion of the sexual glands, water, sodium and potassium excretion from the kidneys, initiation and continuity of lactation, as well as mammary gland growth and lactogenesis (Tucker 1974; Collier *et al.,* 1984; Horseman *et al.,* 1997). For males, it contributes to the continuation of normal testosterone production in physiological doses and have more than 300 effects, such as affecting sperm motility and fertility (Horseman *et al*., 1997). For these reasons, *PRL* gene may be an excellent candidate gene for use in breeding livestock.

RsaI endonuclease polymorphism have been identified by using PCR-RFLP with Genetic polymorphism studies on cattle *PRL* gene sequences (Mitra *et al.,* 1995; Brym *et al.,* 2005). The statistical relationships between these polymorphic variants and milk yield traits in cattle were examined by many researchers and some significant associations were reported (Table 1). In these researches, *RsaI*$^{-}$ allele gene frequency of *prolactin* gene polymorphism was found to be higher than *RsaI*$^{+}$ allele gene frequency. It has also been suggested in the majority of researches that *Prolactin* variants may be useful in direct breeding programs to improve milk traits in animals (Alipanah *et al.,* 2008; Rorie *et al.,* 2009; Akyuz *et al.,* 2012; Alfonso *et al.,* 2012; Boleckova *et al.,* 2012; Gayari *et al.,* 2020). Generally, the results show that the *PRL*/RsaI$^{(-)}$ allele is unfavourable for milk and protein yield, but for fat yield the *PRL*/RsaI$^{(+)}$ allele effect is significant for milk and protein yield. In adition to, in literature, it is seen that *PRL*/RsaI$^{(+/-)}$ polymorphism is reported as A/B or A/G (genotypes; GG=AA=*RsaI*$^{-/-}$, AG=AB=*RsaI*$^{-/+}$, AA=BB=*RsaI*$^{+/+}$) (Ozdemir 2020).

Table 1. *PRL/RsaI* gene polymorhism belonging to various cattle breeds and its associations with some production traits.

References	Breeds	*RsaI*$^{(-)}$	*RsaI*$^{(+)}$	Its associated production traits
Mitra *et al.,* 1995	German Black Pied	0.80	0.20	-
	Swiss Brown	0.61	0.39	
	Sahiwal	0.49	0.51	
Dybus, 2002	Polish	0.86	0.14	Milk yield
Dybus *et al.,* 2005	Black and white	0.85	0.15	Fat yield
	Jersey	0.31	0.69	
Brym *et al.,* 2005	Black and White	0.89	0.11	Milk and fat yield
	Jersey	0.29	0.71	
Miceikiene *et al.,* 2006	Lithuanian dairy cattle breeds	0.87	0.13	Milk, fat and protein yield, fat and protein ratio
Alipanah *et al.,* 2008	Rusian Black	0.71	0.29	Milk and fat yield
	Rusian Red	0.70	0.30	
Oztabak *et al.,* 2008	East Anatolian Red	0.56	0.44	Milk yield
	South Anatolian Red	0.74	0.26	
Mehmannavaz *et al.,* 2009	Holstein bulls	0.93	0.07	Milk, fat and protein yield
Rorie *et al.,* 2009	Holstein	0.92	0.08	Milk yield and Mastitis
	Crossbreed	0.70	0.30	
Kaplan & Boztepe, 2010	Brown Swiss	0.82	0.18	-
	Anatolian buffalo	1.00	0.00	
Akyuz *et al.,* 2012	Turkish Gray	0.76	0.24	-
	East Anatolian Red	0.70	0.30	
	Anatolian Black	0.58	0.42	
	South Anatolian Red	0.76	0.24	
	Brown Swiss	0.73	0.27	
	Holstein	0.86	0.14	
Alfonso *et al.,* 2012	American Swiss	0.88	0.12	Milk yield
Boleckova *et al*., 2012	Fleckvieh	0.88	0.12	Milk, fat and protein yield
Mahajan *et al.,* 2012	Frieswal	0.63	0.37	Lactation length and service period
Bukhari *et al.,* 2013	Frieswal	0.63	0.37	
Akyuz and Cinar, 2014	East Anatolian Red	0.74	0.26	-
	Brown Swiss	0.44	0.56	
	Zavot	0.65	0.35	
	Simmental	0.67	0.33	
Ozkan Unal *et al.,* 2015	Turkish Gray	0.70	0.30	-
	East Anatolian Red	0.68	0.32	
	Anatolian Black	0.52	0.48	
	Sout Anatolian Red	0.71	0.29	
Sonmez and Ozdemir, 2017	East Anatolian Red	0.76	0.24	-
Gayari *et al.,* 2020	Crossbreed	0.53	0.47	Milk yield, Sexual maturity and First calving age

The aim of this study was to ascertain the *RsaI* polymorphism of the *Prolactin* (*PRL*) gene of 186 organically reared Holstein cattle by PCR-RFLP method, in order to reveal the genetic variation of the population, and to reveal the relationship between *PRL*/*RsaI* genotypes and some milk yield traits.

MATERIALS AND METHODS

In the present study, 186 Holstein cattle reared in the Kelkit region of Gumushane were used as material. Genomic DNA was obtained by commercial DNA isolation kit (Purgene DNA kit (Gentra Systems, Minnesota, USA)). PCR was performed to replicate the related gene region of the obtained DNAs, qualitative and quantitative controls of the analysis results were carried out by 2% agarose gel electrophoresis.

PRL/*RsaI* Forward:5'-TTC ATG AAG CTG CTC ACC TG-3',Revers:5'-TTG ATT CTT GGG TTG CTG CG-3' primers (Sonmez and Ozdemir, 2017) were used in the analysis. For PCR amplification, to complete a final volume of 20 µl, about 50-100 ng genomic DNA, Buffer (pH: 8.5) 5.0 µl (10X), F Primer; 10 pmol/µl, R Primer; 10 pmol/µl, MgCl2; 1.2 µl, Taq; 0.5-1.0 units, dNTP; 2.5 µl assembled and with ddH2O the total volume was supplemented to 20 µl. At the first step of the PCR cycle conditions denaturation for the examined *PRL* gene region were programmed as 1 cycle at 94°C for 5 min.; at the second step including 30 cycles at 5'UTR and 4th exon, for 1 cycle consisting of 94°C for 45 sec and 30 sec, annealing temperatures of 58°C for 45 sec and 61°C for 48 sec; initial extension temperatures of 72°C for 45 sec, and 40 sec; the final extension temperatures of 72°C for 5 min, respectively.

Approximately 8-10 µl of each amplified sample was performed in 0.2 ml sterilized eppendorf tubes and restriction enzyme for 2-5 U related region, 2-5 µl RE buffer, 5 µl ddH$_2$O were added on each sample and sealed with 10-15 µl mineral oil. Then placed into the etuve, the incubation process was performed at 37 ° C for 12 hours.

GenAlEx 6.5 (Peakall and Smouse, 2012) program was used for H-W genetic equilibrium test of genotype frequencies and allele gene and genotype frequencies of *PRL* locus of examined Holstein cattle. The SPSS 20.0 statistics package software (IBM Corp. Released 2011. IBM SPSS Statistics for Windows, Version 20.0. Armonk, NY: IBM Corp.) was used in case of evaluating the yield records of the obtained polymorphic systems, and mean separation procedures were performed using a Duncan multiple range test.

In the study conducted to test the effect of the *prolactin* genotypes belonging to cattle obtained at different periods on some milk yield performance, the following general linear model was adopted based on least squares method.

$Y_{ijk} = \mu + a_i + b_j + c_k + e_{ijkl}$

Where,

Y_{ijk}; the evaluated response variable (Lactation milk yield, 305d milk yield, Peak daily milk and Lactation length), µ; expected mean, a_i; the effect of i. genotype (*RsaI*$^{-/-}$, *RsaI*$^{-/+}$, *RsaI*$^{+/+}$, b_j; the effect of the order of j. lactation (1, 2, 3, 4), c_k; the effect of k. open days number (1:≤60d, 2:61-90d, 3: 91-120d, 4:121-150d and 5≥151d) and e_{ijkl}; the random error.

RESULTS AND DISCUSSION

In the present study, 4th exon of the *PRL* gene was obtained in three bands of 210/120/90 bp by cutting 210 bp PCR product with the *RsaI* enzyme. The identification and cutting regions of the restriction enzyme and the number and size of the bands of the genotypes are presented in Table 2.

Table 2. Cutting region and fragments of *RsaI* restriction enzymes on *Prolactin* gene.

PCR product (bp)	Recognition sequence (5′→3′)	Genotype and fragment size (bp)
210	GT^AC	RsaI$^{-/-}$ =210 RsaI$^{+/+}$ =120, 90 RsaI$^{-/+}$ =210, 120, 90

As a result of PCR-RFLP analysis, a total of 186 animals were genotyped successfully and in the polymorphic region of the *PRL* gene, the frequencies of *RsaI*$^{-/-}$, *RsaI*$^{-/+}$ and *RsaI*$^{+/+}$ genotypes were determined as 0.26, 0.52 and 0.22, respectively. In the population examined, the 4th exon *RsaI*$^{-}$allele gene frequency was 0.52 and the *RsaI*$^{+}$ allele gene frequency was 0.48 (Table 3).

The most common genotype frequency of the exon 4 is the *RsaI*$^{-/+}$ genotype and the value is 0.52. The lowest genotype frequency has been the *RsaI*$^{+/+}$ genotype in population.

In all of the studies, including previous studies with different breeds (Table 1) to determine the polymorphism in the *Rsal* polymorphic region of the *PRL* gene, performed to determine *PRL* polymorphism, the *RsaI*$^{-}$ allele frequency was found to be high and the frequency of the *RsaI*$^{+}$ low and seemed to be in accordance with our findings (Table 1).

According to the Hardy-Weinberg genetic balance test performed, the distribution of genotype frequencies was seen to be in equilibrium ($P>0.05$) (Table 4).. Least square means and standard errors for lactation milk yield, 305d milk yield, peak milk yield and lactation length of *PRL* genotypes are shown in Table 5.

Table 3. Genotype and allele frequencies of *PRL* gene belonging to Holstein population.

Genotype			Allele Frequency	
RsaI$^{-/-}$	*RsaI*$^{-/+}$	*RsaI*$^{+/+}$	*RsaI*$^{-}$	*RsaI*$^{+}$
0.26	0.52	0.22	0.52	0.48

Table 4. Genotype Frequencies of *PRL/RsaI* polymorphism and H-W Genetic Test

N	Observed			Expected			(X^2 Test)
	RsaI$^{-/-}$	*RsaI*$^{-/+}$	*RsaI*$^{+/+}$	*RsaI*$^{-/-}$	*RsaI*$^{-/+}$	*RsaI*$^{+/+}$	
186	49	96	41	50	93	43	0.22 ns

ns:$P>0.05$.

Table 5. Least Squares Means and Standard Errors For Lactation Milk Yield, 305d Milk Yield, Peak Daily Milk and Lactation Length.

Trait Effect		N	Lactation Milk Yield (Kg)	305d Milk Yield (Kg)	Peak Daily Milk (Kg)	Lactation Length (d)
***PRL* Genotype**	***RsaI***$^{-/-}$	102	7190,5±269,0	7281,5±209,1	35,4±0,7^{a*}	318,7±10,6
	RsaI$^{-/+}$	287	6989,2±189,0	7257,1±143,5	35,5±0,5^{a}	313,2±7,3
	RsaI$^{+/+}$	141	7479.0±245,9	7543,3±179,1	37,1±0,6^{b}	317,4±9,7
Lactation Parity	**1**	195	6110,4±204,2^{a**}	6579,15±213,2^{a**}	31,6±0,5^{a**}	290,8±8,2^{b**}
	2	195	7825,7±193,6^{b}	7721,2±133,9^{b}	35,2±0,5^{b}	328,2±7,8^{c}
	3	105	8612,9±259,9^{c}	7570,0±179,0^{b}	38,0±0,6^{c}	390,3±9,0^{d}
	4	35	6329,4±438,1^{a}	7572,273±300,0^{b}	39,7±1,2^{d}	256,5±17,6^{a}
Open Days	**1**	138	7791,5±232,6^{c**}	7077,9±157,7^{a**}	35,7±0,6^{ab*}	391,9±9,3^{e**}
	2	31	7655,8±445,1^{c}	7197,4±325,1^{ab}	35,6±1,1^{ab}	350,7±17,3^{d}
	3	40	7071,2±398,3^{b}	7023,1±295,5^{a}	35,1±0,9^{a}	306,3±15,2^{c}
	4	61	7087,2±328,0^{b}	7489,6±288,6^{ab}	36,9±0,8b^{c}	281,5±12,9^{b}
	≥5	260	6492,4±168, 5^{a}	8015,3±130,4^{c}	37,5±0,5^{c}	251,8±6,5^{a}
Overall		530	7219,6±174,9	7360,7±132,1	36,1±0,5	316,5±6,9

Means in columns with different superscripts are significantly different. $^{*}P<0.05$,$^{**}P<0.01$.

There was no significant association between lactation milk yield, 305 day milk yield and lactation length milk yield with the detected *PRL* genotypes, only a significant association was found for peak milk yield (P<0.05) (Table 5). In general, it appears that *PRL*/*RsaI* polymorphism is associated with milk yield traits in cattle and therefore is not in line with the results of other published studies (Table 1). However, due to the genotype environment interactions or from the samples conducted the results may differ.

The different results reported in the literature and in this study show that *PRL*/*RsaI* polymorphism is not directly accountable for phenotypic variations and these contradictions can be explained by differences in genetic linkage balance as well as genotype, breed and environment interactions. Nevertheless, further investigation is necessary to determine whether there is an association between *PRL*/*RsaI* genotypes and milk traits in different cattle breeds that were reared in various environments.

A successful breeding study in terms of various yield traits in animals can be achieved by first revealing the genetic structure in a good way. Researches in genetic structure determination, significant distances have been covered in recent years over direct DNA techniques. Nowadays, the aim of most researchers is to use new techniques at the molecular level to help both breeding their countries' breeds and protect them as a native gene resource and to produce detailed results about the genetic structure of various breeds at the DNA level studies.

Polymorphisms, unlike mutations, do not cause dysfunction in the organism and do not cause a pathology. Since the polymorphisms in the cattle genome are at a high size, the size and qualities of these polymorphic structures must be determined on a preferential basis accurately and detailed in terms of breeding.

Conclusıon: The results obtained in the study can be considered satisfactory in terms of revealing Holstein breeds *prolactin* polymorphism. However, in subsequent researches, it is necessary to carry out a larger number of sampling to replicate researches, and to associate polymorphic structures of *prolactin* gene with different yield traits of the animals.

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