The Determination of Prolactin Gene Polymorphism Using PCR-RFLP Method within Indigenous Anatolian Water Buffalo and Brown Swiss

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Abstract: The objective of this research was to determine the prolactin gene polymorphism within Indigenous Anatolian Water Buffalo breed and Brown Swiss cattle by using PCR-RFLP method. Experimental material for this study consists of 45 Indigenous Anatolian Water Buffalo breed and 30 Brown Swiss cattle. According to the research evidence, Indigenous Anatolian Water Buffalo breed was monomorphic at the exon 3 *PRL-Rsa*I loci. On the other hand, the polymorphism association with exon 3 *PRL-Rsa*I loci detected in Brown Swiss cattle. The allelic frequencies (A, B) in Brown Swiss cattle were 0.82 and 0.18, respectively. The genotype frequencies of AA and AB were 0.63 and 0.37, respectively. The BB genotype was not found in the present study.

Keywords: Indigenous Anatolian Water Buffalo, Brown Swiss, prolactin, polymorphism, PCR-RFLP

Introduction

Nowadays, molecular methods such as RAPD, RFLP, AFLP, STS, STR, SNP have been increasing in animal breeding. To determine the specific genes concerning with economic characteristics of farm animals; first of all candidate genes involved in those should be studied. Some genes are shown as candidates associated with milk yield and characteristics for marker assist selections (*MAS*) in dairy cattle. As a matter of fact that prolactin affects not only improving mammary glands, but also initiation and maintenance of lactation. Having all these influences and qualities, it comes foreground among the candidate genes.

Prolactin as the first pituitary gland hormone was purified and identified nearly 80 years ago. Prolactin was named as pro–lactin because of the stimulatory effects on lactation and mammary gland development. Prolactin is known to have more than 300 biological activities such as water and electrolyte balance, growth and development, immune and reproductive function (Gregerson, 2006). It has been identified that prolactin is secreted in many different places as neurons, prostate, mammary epithelial, endothelial cells and skin cell (Lastra et al., 2002).

The number amino acid of prolactin, which is a hormone structure of polypeptide, changes with regard to the species of the living organisms. For example, while prolactin consists of 197 amino acid in rats and mice; it consists of 199 amino acid in human, sheep, cattle and pigs (Freeman et al., 2000). Prolactin is located on chromosome 23 in cattle and comprised of 5 exons and 4 introns (Dybus et al., 2005).

There are many studies in relation to prolactin gene polymorphism. A significant portion of these works is composed of RFLP and SSCP mutations (Brym et al., 2005). As an example of these mutations, Mitra et al., (1995) detected the exon 3 A-G point mutation

The aim of this study was to determine the exon 3 prolactin gene polymorphisms both Indigenous Anatolian Water Buffalo breed, which is covered the gene resources conservation program for decreasing

numbers in Turkey, and Brown Swiss cattle, which has an important place in the existence of culture breed cattle.

Material and Method

Material and DNA Isolation

Blood samples for DNA isolation for Indigenous Anatolian Water Buffalo breed were collected from Amasya, Afyon, Konya and Sivas provinces in Turkey. Brown Swiss blood samples were provided from Konuklar State Farm in Konya province. Genomic DNAs was extracted from blood samples using salting out technique with slightly modifications (Miller, 1998).

PCR Amplification

Detection of restriction fragment length polymorphism (RFLP) was carried out according to Mitra et al., (1995). The 156-bp fragment of the exon 3 *PRL-RsaI* loci was amplified using following primers: forward:5'- CGA GTC CTT ATG AGC TTG ATT CTT -3', and reverse: 5'- GCC TTC CAG AAG TCG TTT GTT TTC -3'. The PCR was performed in a reaction volume of 15 µl containing approximately 50-100 ng of genomic DNA, 1x Buffer (pH: 8.5), 1.5 mM $MgCl_2$ (supplied with the enzyme), 0.27 µM of each primer, 0.25 µM dNTPs and 0.4 units of Taq polymerase (*Fermantas*) with 9.48 ul sterile distilled water. The PCR application conducted in Thermal Cycler (Techne TC-512). The PCR conditions included initial denaturation step for 2 min at 94°C, followed by 35 cycles with denaturation at 94°C for 45 sec; annealing at 60°C for 45 sec; extention at 72°C for 1 min and final exention for 5 min at 72°C. The amplified DNA fragment of the exon 3 *PRL* loci was digested at 37°C for overnight with *Rsa*I (*5 U/ul, Fermantas*). The digestion products were separated on 3% Prona agarose gel (Nu microphor) in 1x TRIS-borate-EDTA (TBE) buffer. The gel was stained with ethidium bromide and visualized in gel documentation system under UV light by means of transilluminator.

Statistical Analysis

Statistical analysis was carried out by PopGene Version 1.32 (Yeh et al., 1997)**.** The *Chi*-square test was used to evaluate whether the population was Hardy-Weinberg equilibrium (Düzgüneş et al., 1983).

Results and Discussion

As a result of this study prolactin gene exon 3 *Rsa* I digestion in Indigenous Water buffalo and Brown Swiss cattle is shown in Figure 1. The genotype and allele frequencies at *PRL-Rsa*I loci in Brown Swiss cattle are shown in Table 1.

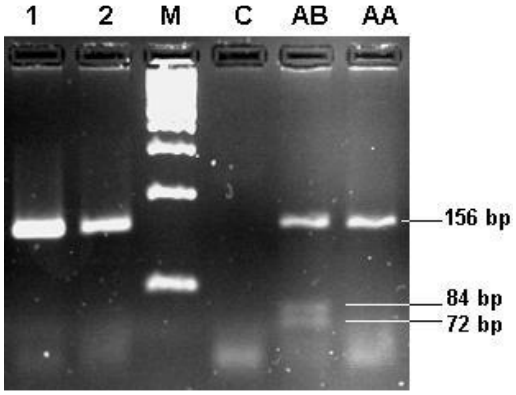

Figure 1. Restriction analysis of *PRL* 156 – bp PCR products digested with *Rsa* I on 3% Prona agarose gel (Nu microphor) electrophoresis stained with ethidium bromide. 1 – 2: PCR products, M: 100 bp ladder marker, C: Control, AB: *Rsa* I digested PCR product (156, 82, 74 bp) and AA: Undigested PCR product.

*PRL-Rsa*I loci	N	Genotypes			Allel frequency		Genotype frequency			(χ^2)[1]
		AA	AB	BB	A	B	AA	AB	BB	
Observed	30	19	11	0	0.82	0.18	0.63	0.37	0.00	1.356293 Ns
Expected	*30*	*20.172*	*8.856*	*0.972*			*0.67*	*0.30*	*0.03*	*0.557* Ns

[1]Test of Hardy-Weinberg equilibrium; Ns: not significant. (P > 0.05)

Table 1. The genotype and allele frequencies at exon 3 *PRL-Rsa*I loci in Brown Swiss cattle

Table 1 shows the allelic frequencies (A, B) in Brown Swiss cattle were 0.82 and 0.18, respectively. The genotype frequencies of AA and AB were 0.63 and 0.37, respectively. The BB genotype was not found in the present study. According to the *Chi*-square test, the result of Brown Swiss cattle population has emerged in Hardy-Weinberg equilibrium. The recent studies with regard to exon 3 *PRL-Rsa*I loci in buffalo breed and cattle are presented in Table 2.

Source	Buffalo	Cattle	N	Allele Frequencies (%)		R
				A	B	
Alipanah et al., 2007		Holstein	72	0.71	0.29	AB*
Skinkytė et al., 2005		Holstein	52	0.79	0.11	α
Maksymiec et al., 2008		Holstein	720	0.58	0.41	α
Udina et al., 2001		Holstein	23	0.80	0.20	α
Dybus et al., 2005		Holstein	242	0.85	0.14	α
Kumari et al., 2008		Holstein	223	0.90	0.10	α
Miceikienė et al., 2006		Holstein	109	0.80	0.20	α
Khatami et al., 2005		Holstein	32	0.98	0.05	α
Dybus et al., 2005		Jersey	185	0.30	0.69	α
Kumari et al., 2008		Jersey	143	0.55	0.45	α
Skinkytė et al., 2005		Lithuanian Red	136	0.87	0.13	α
Öztabak et al., 2008		South Anatolian Red	40	0.74	0.26	α
Miceikienė et al., 2006		Lithuanian Red	168	0.77	0.23	α
Udina et al., 2001		Russian Gorbatov Red	35	0.91	0.8	α
Öztabak et al., 2008		East Anatolian Red	40	0.56	0.44	α
Mitra et al., 1995		Sahiwal	57	0.51	0.49	α
Kumari et al., 2008		Sahiwal	13	0.88	0.12	α
Kumari et al., 2008		Kankrej	26	0.60	0.40	α
Sacravarty et al., 2008		Kankrej	57	0.51	0.49	BB*
Mitra et al., 1995		Holstein	23	0.80	0.20	α
Khatami et al., 2005		Holstein	32	0.61	0.39	α
Ghasemi et al., 2009		Montbeliarde	120	0.89	0.11	AA*
Alipanah et al., 2007		Red Holstein	98	0.79	0.21	BB*
Udina et al., 2001		Russian Aryshire	46	0.85	0.14	α
Khatami et al., 2005		Yoroslav	120	0.65	0.35	α
Mitra et al., 1995	Murrah		53	0.93	0.07	α
Mitra et al., 1995	Nili-Ravi		19	0.84	0.16	α
Ladani et al., 2003	Mehsani		44	0.50	0.50	α

Ladani et al., 2003	Surti	30	0.48	0.52	α
Ladani et al., 2003	Jaffarabadi	23	0.43	0.57	α
Average			0.73	0.27	

R: The relationship between genotype and milk yield; * Significant relationship between genotype and milk yield; α: Not estimated relationship between genotype and milk yield

Table 2. The recent studies with regard to exon 3 *PRL-Rsa*I loci in buffalo breed and cattle

As it can be seen from Table 2, there is no literature encountered with reference to exon 3 *PRL-Rsa*I loci polymorphism in Brown Swiss cattle.

As for the Holstein cattle, which is known as high milk yield over the world, the highest frequency of allele A (0.98) in the analysed population of Holstein cattle reported by Khatami et al., (2005). On the other hand the smallest frequency of allele A (0.58) reported by Maksymiec et al., (2008). In addition, Alipanah et al., (2007) reported that AB genotype breed in Holstein cattle have higher milk yield than AA and BB genotype.

In this study, we found exon 3 *PRL-Rsa*I loci polymorphism in 11 of 30 Brown Swiss cattle. The frequencies of exon 3 *PRL-Rsa*I alleles were found as follows; A (0.82), B (0.18) in Brown Swiss cattle. The average frequencies of A and B allele in literature were 0.73 and 0.27, respectively. The frequencies of allele's exon 3 *PRL-Rsa*I in this study were identical in comparison with literature. As for the genotype frequencies, AA, AB and BB genotypes were found 0.63, 0.37 and 0.00, respectively. According to the results of studies in Table 2, it is clear that the frequency of B allele is quite low. Because of these results, BB genotype is quite low too.

Another result of in this study, Indigenous Anatolian Water Buffalo breed was monomorphic at the exon 3 *PRL-Rsa*I loci. However, Mitra et al., (1995) reported that the allelic frequency of A was found as 0.93 and 0.84, respectively in Murrah and Nili Ravi buffalo breed. In addition, the study made by Ladani et al., (2003) as to exon 3 *PRL-Rsa*I loci in Mehsani, Surti and Jaffarabadi buffalo breed, the frequencies of A allele was found 0.50, 0.48 and 0.43, respectively.

As mentioned previously in this study, prolactin gene mutation in exon 3 *Rsa*I digestion site is not observed 45 Indigenous Anatolian Water Buffalo breed. This result is identical to the results have been reported by Mitra et al., (1995). These researchers who carried out the study about exon 3 *PRL-Rsa*I loci in Murrah, Nili Ravi and Egypt buffalo breed stated that they have observed mutations in Murrah and Nili Ravi buffalo breed, whereas they have not observed any mutation in Egypt buffalo breed. As a result of this study, they have concluded that identified prolactin gene mutations in exon 3 *PRL-Rsa*I loci may vary according to the type of buffalo. Yet, these interestingly enough, mutations couldn't be detected in some buffalo species. Hence, we thought that couldn't be found any mutation in exon 3 *PRL-Rsa*I loci in Indigenous Anatolian Water Buffalo breed may be a result of this situation.

Conclusion

At the end of the recent studies, some researchers have emphasized that there is a strict relationship between genotypes and milk yield. Some researchers (Alipanah et al., 2007; Ghasemi et al., 2009 and Sacravarty et al., 2008) claimed that AB genotype in Holstein cattle has higher milk yield than other genotypes. Alipanah et al., (2007) stated that AA genotype cattle has higher milk yield in Montbeliarde cattle. Ghasemi et al., (2009) proposed BB genotype in Kankrej cattle. However, these studies in question have a small place in all of the study. Consequently, in order to have more concrete results and more sound decisions about prolactin gene further investigations should be done due to the fact that prolactin has a significant influence on lactation and mammary gland development.

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