Association of prolactin gene variants with milk production traits in Russian Red Pied cattle

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Abstract
A total of 125 Russian Red Pied cows were genotyped for the prolactin-related gene. The PRL-RsaI genotypes were analysed using the Polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method. In this breed, the frequencies of alleles were as follows; A= 0.794 and B= 0.206. The frequencies of AA, AB and BB genotypes were 0.598, 0.392 and 0.01, respectively. Results showed that: BB genotype had higher milk yield than AA and AB individuals (P< 0.05). BB genotype showed higher milk fat yield than AA and AB individuals (P< 0.05). With respect to milk fat content (%), the AB genotype had higher levels than the AA and BB individuals (P< 0.05). No differences between the cows of different PRL-RsaI genotypes were found in terms of milk fat yield and milk protein concentration. The results showed that the highest milk and milk fat yields were obtained by cows with the genotype PRL-RsaI BB. The results presented here demonstrate that the prolactin gene may be considered as a marker for dairy traits in cattle.

Keywords: Prolactin; Polymorphism; Cattle; PCR-RFLP; Milk production; Red Pied.

INTRODUCTION
Many genes are involved in milk production. Among them, caseins are the major constituents of total milk proteins. In bovines, caseins genes are located within a 200-kb region on chromosome 6 (Ferretti et al., 1990; Threadgill and Womack, 1990). Several DNA polymorphisms have been found for each casein gene, most of them based on previously described protein variants (Eigel et al., 1984). In addition, the prolactin hormone is responsible not only for triggering lactation but also for mammary gland growth and lactogenesis (Tucker., 1981; Collier et al., 1984). This feature suggests that this locus might be used as a genetic marker for milk production.

Prolactin (PRL) is one of the most versatile hormones of the pituitary gland in terms of its biological activities. More than 100 different and distinct effects of this hormone have been documented. Prolactin is essential for the initiation and maintenance of lactation, being also primarily responsible for the synthesis of milk proteins, lactose, lipids and all other major components of milk (Le Provost et al., 1994).

Prolactin is a polypeptide hormone with multiple functions, secreted mainly by the anterior pituitary gland (Bole-Feyssot et al., 1998). Gene disruption experiments have proved their mandatory role in mammary gland development, lactogenesis, and expression of milk protein genes (Horseman et al., 1997). Therefore the bovine prolactin gene (PRL) seems to be an excellent candidate for linkage analysis of quantitative trait loci (QTL) affecting milk production traits.

Within the bovine PRL gene, several polymorphisms have been reported (Cowan et al., 1989; Hart et al., 1993; Zhang et al., 1994; Chung and Kim 1997). On the basis of sequence analysis of four different cDNA clones, seven possible nucleotide substitutions were described by Sasavage et al. (1982). One of them, recognized by the RsaI endonuclease, has become a popular genetic marker used for genetic characterization of cattle populations by means of PCR-RFLP (Mitra et al., 1995; Chrenek et al., 1998;
Udina et al., 2001; Dybus, 2001; Kaczynski, 2002). Two allelic variants (B and b) have been distinguished at the DNA level, based on Rsal polymorphism in the third exon of the coding region. It has been suggested that prolactin alleles correlate with milk yield (Lewin et al., 1992). This marker has also been used for the initiation of studies on possible associations between prolactin gene variants and milk performance traits (Chung et al., 1996; Dybus, 2002).

Prolactin plays an important regulatory function in mammary gland development, milk secretion, and expression of milk protein genes. Hence the PRL gene is a potential genetic marker for production traits in dairy cattle. The gene has been mapped on chromosome 23 by Hallerman et al. (1988). It consists of five exons and four introns (Camper et al., 1984) encoding the 199-amino-acid mature protein (Wallis, 1974). On the basis of sequence analysis of four different cDNA clones, seven possible nucleotide substitutions have been described by Sasavage et al. (1982). One of them, recognized by Rsal endonuclease, has become a popular genetic marker used for genetic characterization of cattle populations by means of PCR-RFLP (Mitra et al., 1995).

The objectives of this work were to study gene frequencies at the prolactin locus, and association of genetic variants of the prolactin gene with milk production traits in Russian Red Pied cattle.

The Red Pied cattle breeds were created via the crossing of Simmental cows with the bulls of the Red Pied Holstein breed. The Russian Red Pied cows, with a population of approximately 100,000 animals, were mainly raised in the Krasnodar, Stavropol, Omsk, Rostov states and other western states of Russia.

**MATERIALS AND METHODS**

A total of 125 Red Pied cows were genotyped for the prolactin gene. The cows were kept in the Drodjba herd in the Varonedj state of Russia. Only cows with complete lactations were included in the statistical analysis. The PRL-Rsal genotypes were analyzed using the PCR-RFLP method. PCR products were amplified using primers: forward 5'-CGAGTCCTTATGAGTTGATTCTT-3' and reverse 5'-GCCTTCCAGGTTGTTTGTTTTC-3' primers. Cycles applied were: denaturation at 94°C/5 min, followed by 30 cycles of 94°C/30 s, annealing at 59°C/40 s, extension at 72°C/20 s, and a final extension at 72°C/3 min. PCR conditions were as follows: 2.5 µl 10X PCR buffer (15 mM MgCl₂), 1.5 µl of dNTP-mix (2 mM each), 1.5 µl of primer (100 pmol/µl each), 0.5 U of Taq DNA polymerase (Fermentase, Russia). Amplified DNA was digested with the Rsal enzyme. Digestion products were separated electrophoretically in 4% w/v agarose gel. Frequencies of distribution of alleles within the herds were compared using the Chi-square test. Data for 305-days milk production, including overall yields of milk, milk fat and milk protein, percent of milk fat, percent of milk protein and combined milk fat and milk protein percent were obtained from the farm records. Statistical calculations were performed using SAS procedures. The effect of PRL genotypes on the milk production traits of cows were analysed using the general linear model (GLM) procedure in SAS (SAS Institute, V 6.4, 1986).

**RESULTS**

The following DNA restriction fragments were obtained for the PRL-Rsal polymorphism: 82 and 74 bps for the BB genotype, 156, 82 bp and 74 bps for the AB genotype and 156 bp (no digestion) for the AA genotype (Figure 1).

In this breed the frequencies of alleles were as follows; A = 0.794, B = 0.206. The frequencies of AA, AB and BB genotypes were 0.598, 0.392 and 0.01, respectively and \( \chi^2 = 0.034 \leq 3.84 \). Frequency of the PRL-Rsal allele A obtained in this study were similar to those reported by Mitra et al. (1995) and Chung et al. (1996) 0.80 and 0.73, respectively.

![Figure 1. Restriction analysis of PRL 156-bp PCR products digested with Rsal by 4% w/v agarose gel electrophoresis stained with ethidium bromide. AA genotype= 156 bp; AB genotype= restriction fragments of 156, 82 and 74 bp; BB genotype= restriction fragment of 82 and 74 bp.](www.SID.ir)
Table 1 shows the effect of the PRL-RsAl polymorphism on milk production traits in cows studied.

BB genotype had higher milk yield (+529.76 kg and +1056.61 kg, respectively) than the AA and AB individuals. Chung et al. (1996) and Dybus (2001) reported that cows with the PRL genotype AA produced more milk than BB individuals but Chrenek et al. (1999) did not prove any significant differences between the animals with different genotypes.

Differences (P ≤ 0.05) between the cows with different PRL-RsAl genotypes for milk fat yield were observed. BB cows showed higher milk fat yields (+12.74 kg and +23.49 kg, respectively) than AA and AB individuals. AA cows yielded more milk fat (+10.75 kg) than AB animals.

There was no significant difference between fat content of the cows with different PRL-RsAl genotypes. But the AB genotype had higher fat contents (+0.13% and +0.08%, respectively) than the AA and BB individuals.

Significant differences between the cows of different PRL-RsAl genotypes were found. But the cows with the BB genotypes produced more milk protein (by 31.14 kg and 38.52 kg, respectively) than AA and AB individuals and cows with the AA genotype produced more milk protein (+7.38 kg) than cows with the AB genotype.

No differences were found in protein content between the cows of different PRL-RsAl genotypes. AA cows produced milk with higher protein content than BB and AB individuals (0.04% and 0.09%, respectively). 

**DISCUSSION**

The study of candidate genes is one of the primary methods to determine whether specific genes are related to economic traits in farm animals. In marker-assisted selection of dairy cattle, some genes are proposed as potential candidates associated with dairy performance traits. Among the various candidates, the prolactin gene seems to be promising, because it plays a crucial role in mammary gland development and in the initiation and maintenance of lactation and expression of milk protein genes. Allelic variation in the structural or regulatory sequences of the prolactin gene would be of interest because of the possible direct or indirect effect on milk production. It may also influence the chemical composition of milk or at least be an effective DNA marker in dairy cattle selection.

Our results showed that the highest milk, milk fat yield and milk protein yield were obtained by cows with the genotype PRL-RsAl BB. Differences results for milk and milk fat were reported by khatami et al. (2005), Brym et al. (2005), Dybus (2001) and Chung et al. (1997) who found that cows with the PRL genotypes AA and AB yielded more milk fat than BB animals. On the other hand, the results by Dybus (2002) for protein content showed that AA cows produced milk with higher protein than AB and BB individuals.

No differences were found between the cows for milk fat content (%) and milk protein content (%). Similar results were reported by Dybus (2001) for milk fat content (%) and by Chrenek et al., (1999) and Chung et al., (1996) for milk protein content (%).

Our results for milk yield were differ of other reported, may be cows with BB genotype were very low frequencies (1%) if no consider cows with BB genotype. Our results exactly are in agreement with results obtained by others that AA cows produced more milk, milk fat content and milk protein content, respectively.

The results presented here show that the PRL gene may be considered as a marker for dairy traits in cattle and it seems that further studies are necessary to implement the use of haplotypes (at least two SNPs within a single locus) which can be used as more informative markers in association studies.

**References**


Prolactin (PRL) and its receptor: actions signal transduction pathways and phenotypes observed in PRL receptor knockout.


