

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-Feysot *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;

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Udina *et al.*, 2001; Dybus, 2001; Kaczynski, 2002). Two allelic variants (*B* and *b*) have been distinguished at the DNA level, based on *RsaI* 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 *RsaI* 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-RsaI* genotypes were analyzed using the PCR-RFLP method. PCR products were amplified using primers: forward 5'-CGAGTCCTTATGAGC TTGATTCTT-3') and reverse (5'-GCCTTCCAGAA GTCGTTTGTTC-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 *RsaI* 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-RsaI* polymorphism: 82 and 74 bps for the *BB* genotype, 156, 82 bp and 74 bps for the *AB* 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-RsaI* 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.

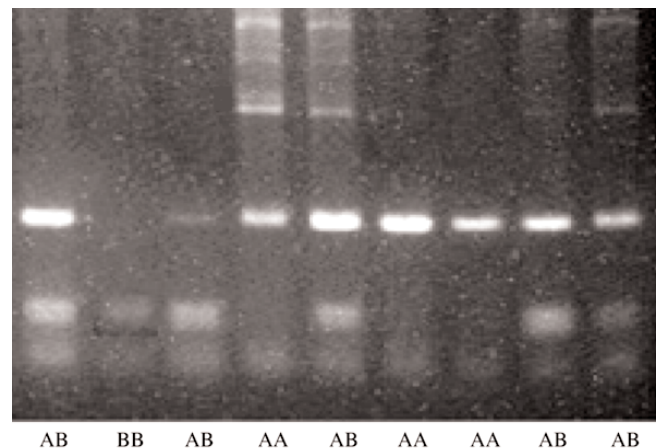


Figure 1. Restriction analysis of *PRL* 156-bp PCR products digested with *RsaI* by 4% w/v agarose gel electrophoresis stained with ethidium bromide. *AA* genotype= 156 bp; *AB* genotype= restriction fragments of 156, 82 and 74 bp; *BB* genotype= restriction fragment of 82 and 74 bp.

Table 1. Effect of Prolactin genotypes on milk traits in Russian Red-Pied cows.

Genotype	Fat% \pm SD	Protein% \pm SD	Fat(kg) \pm SD	Protein(kg) \pm SD	Milk(kg) \pm SD
AA	3,58 \pm 0,63	3,27 \pm 0,09	247,29 ^a \pm 46,30	248,55 ^a \pm 26,56	6709,24 ^a \pm 1328
AB	3,71 \pm 0,52	3,18 \pm 0,60	236,54 ^b \pm 55,92	241,17 ^a \pm 28,88	6182,38 ^b \pm 1511
BB	3,63 \pm 0,39	3,23 \pm 0,01	260,03 ^c \pm 35,65	279,69 ^b \pm 10,00	7239,00 ^c \pm 1504

^{ab} Within columns, means marked by the same superscripts do not differ each other significantly at $P \leq 0.05$.

Table 1 shows the effect of the *PRL-RsaI* 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 ($P \leq 0.05$) between the cows with different *PRL-RsaI* 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-RsaI* 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-RsaI* 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-RsaI* 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-RsaI 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.

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