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Dairy productivity genetic potential of holstein and brown breeds Sires in Kazakhstan

Abstract

The research focuses on the identification of desirable gene's alleles responsible for milk quality and production. DNA was extracted from 11 blood samples of 5 Holstein ("Sayram" local ecotype) and 6 Brown breed ("Ak-Yrys" local ecotype) for identification and genotyping of kappa-casein, thyroglobulin, prolactin and growth hormone genes by polymerase chain reaction restriction fragment length polymorphism (PCR-RFLP) assay. The frequency for GG, GA and AA genotypes of prolactin gene was found to be 0.091, 0.182 and 0.727, respectively. The allele frequency for G and A was 0.901 and 0.081, respectively. Frequencies for BB, AB and AA genotypes of kappa-casein were 0.091, 0.182 and 0.727, respectively. The allele frequency for A and B was 0.901 and 0.081, respectively. The frequency for CC, CT and TT genotypes of thyroglobulin gene was found to be 0.636, 0.273 and 0.088, respectively. The allele frequency for C and T was 0.901 and 0.081, respectively. The frequency for VV, VL and LL genotypes of growth hormone gene were 0.545, 0.366 and 0.089, respectively. The allele frequency for V and L was 0.901 and 0.081, respectively. According to previous studies *GG* genotypes of prolactin gene, BB genotypes of kappa-casein gene, TT genotypes of thyroglobulin and LL genotypes of growth hormone gene are an important factors in increasing milk yield and quality and the results of this study could be used in creating the next generation of valuable animals.

Introduction

The identification of sire's desirable genotypes is an important element in the selection of highly productive animals. The success of breeding is highly dependent on the accurate detection of animal's valuable genotypes. Therefore, identification of best animals and prediction their pedigree qualities at early ages play significant role in the selection process. The achievements of modern molecular genetics can identify genes controlling targeted useful traits of farm animals.

Identification of gene variants is an addition to the traditional animals selection and allows select animals directly at the DNA level. DNA technology allows determining a genotype of an animal regardless gender, age and physiological state, which is an important factor in productivity prediction. Thus, genomic selection of desired genotypes is less time consuming and can significantly reduce expenditures on a creation of high productive herds.

In marker-assisted selection (MAS) of dairy cattle certain genes are proposed as potential candidates. Among them are growth hormone, thyroglobulin, prolactin and kappa- casein genes associated with dairy performance traits.

Allele variants of the genes were distinguished by PCR-RFLP analysis in some areas of the world [1]. The aim of this study was to determine possible kappa- casein, growth hormone, prolactin and thyroglobuline genes polymorphism in Holstein ("Sayram" local ecotype) and Brown ("Ak-Yrys" local ecotype) cattle using PCR-RFLP technique.

Materials and methods

Samples collection and DNA extraction. Blood samples were obtained from 5 Holstein of "Sayram" local ecotype and 6 Brown breed of "Ak-Yrys" local ecotype according to standard instructions [2].

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Blood samples were collected into vacutainer tubes containing sodium EDTA as an anticoagulant.

The tubes were maintained at -20°C until used for DNA extraction. Genomic DNA was extracted from 100 µl blood samples. DNA extraction was performed using DNA isolation kit (Promega Inc., Madison, Wisconsin, USA) according to manufacturer's instructions.

The gel monitoring and spectrophotometer were used for determination of the DNA quality and quantity.

Primer design and synthesis. Design and synthesis of primers for growth hormone (bGH), thyroglobulin (TG), kappa-casein and prolactin genes were conducted using PrimerPlex and DNAMAN software, as well as databases of the National Center for Biotechnology Research (NCBI, USA).

PCR-RFLP assay for growth hormone, thyroglobulin, kappa-casein and prolactin genotypes. Genotyping of the genes was performed using PCR-RFLP. The 208 bp *bGH,* 473bp *TG5,* 453 bp *kCS,* 294 bp *PL* promoter fragments were PCR amplified from genomic DNA. The sequences of primers used for amplification of *bGH* gene containing polymorphic sites for *V* and *L* alleles were: *bGH* F: 5'-ATCCACACCCCCTCCACACAgT -3' and *bGH* R: 5'-CATTTTCCACCCTCCCCTACAg -3'. The sequences of primers used for amplification of *TG5* gene containing polymorphic sites for *C* and *T* alleles were: *TG5* F: 5'-GTGAAAATCTTGTGGAGGCTGTA-3'and *TG5* R: 5'-GGGGATGACTACGAGTATGACTg -3'. The sequences of primers used for amplification of *kCS* gene containing polymorphic sites for *A* and *B* alleles were: *kCS* F: 5'- CACGTCACCCACACCCACATTTATC- 3' and *kCS* R: 5'-TAATTAGCCCATTTCGCCTTCTCTGT- 3'. The sequences of primers used for amplification of *PL* gene containing polymorphic sites for *G* and *L* alleles were: *PL* F: 5'-CCAAATCCACTGAATTATGCTT-3' and *PL* R: 5'-ACAGAAATCACCTCTCTCATTCA-3'

Amplification reactions were done in a final volume of 25 µl, containing 100 ng DNA, 0.4 µM of each primer, 1X PCR buffer, 2.5 mM $MgCl_2$, 200 mM dNTPs and 1U of *Taq* polymerase.

Thermal cycling conditions included: an initial denaturation step at 95°C for 3 min followed by 40 cycles of 95°C for 30sec, 58°C for 30 min, 72°C for 1 min and a final extension for 7 min at 72°C. PCR products were recognized by electrophoresis on 1% agarose gel. The restriction digestion of the PCR products was carried out with *Alu*I enzyme for *bGH*, *BstXI* enzyme for *TG5*, *HindIII* enzyme for *kCS* and *RsaI* enzyme for *PL*.

The PCR products were subjected to digestion by restriction enzymes in a total volume of 20 µl (10 µl PCR product, 2 µl enzyme buffers, 0.5 µl enzymes, and 7.5 µl distilled water) and placed in the incubator at 37°C for 4 h according to manufacturer's instructions (Promega Inc., Madison, Wisconsin, USA).

DNA electrophoresis was performed according to standard protocols [3].

Results and their discussion

The PCR product of the primer specific for kappa casein gene gives the 453 bp DNA fragment. Digestion of 453 bp fragment of kappa-casein gene by *Hind*III restriction endonuclease generated two fragments at 351 and 102 bp sizes.

The results were the 453 bp fragment of uncut PCR product representing homozygotes *A* allele, two fragments of 351 and 102 bp representing homozygotes B allele, and three fragments 453, 351 and 102 bp representing heterozygotes (*AB*) for kappa casein gene.

A representative PCR and RFLP pattern is depicted in Figure 1. On the other hand, the amplified DNA (453 bp) from blood samples were synthesized by *Taq*-pol enzyme (Figure 3).

The PCR product of the primer specific for thyroglobulin gene gives the 473 bp DNA fragment. Digestion of 473 bp fragment of thyroglobulin gene by *BstXI* restriction endonuclease generated two fragments at 295 and 178 bp sizes.

The results were the 473 bp fragment of uncut PCR product representing homozygotes *T* allele, two fragments of 295 and 178 bp representing homozygotes *C* allele, and three fragments 473, 295 and 178 bp representing heterozygotes (*CT*) for thyroglobulin gene.

A representative PCR and RFLP pattern is depicted in Figure 2.

On the other hand, the amplified DNA (473 bp) from blood samples were synthesized by *Taq*-pol enzyme (Figure 3).

The PCR product of the primer specific for growth hormone gene gives the 208 bp DNA fragment.

Digestion of 208 bp fragment of growth hormone gene by *AluI* restriction endonuclease generated two fragments at 173 and 35 bp sizes.

The results were the 208 bp fragment of uncut PCR product representing homozygotes *V* allele, two fragments of 173and 35 bp representing homozygotes *L* allele, and three fragments 208, 173 and 35 bp representing heterozygotes (*VL*) for growth hormone gene. A representative PCR and RFLP pattern is depicted in Figure 4.

The PCR product of the primer specific for prolactin gene gives the 294 bp DNA fragment.

Digestion of 208 bp fragment of prolactin gene by *RsaI* restriction endonuclease generated two fragments at 162 and 132 bp sizes.

The results were the 294 bp fragment of uncut PCR product representing homozygotes *G* allele, two fragments of 162 and 132 bp representing homozygotes *A* allele, and three fragments 294, 162 and 132 bp representing heterozygotes (*GA*) for prolactin.

A representative PCR and RFLP pattern is depicted in Figure 5.

On the other hand, the amplified DNA (473 bp) from blood samples were synthesized by *Taq*-pol enzyme (Figure 6).

The number of genotypes and frequency of alleles of analyzed animals are shown in Table 1.

K-Casein is of special interest as a milk protein due to its relationship with milk quality and composition [4].

Kappa-casein constitutes approximately 12% of the casein and is a constituent of bovine milk [5].

Casein constitutes 80% of total milk protein (cow's milk contains 3 to 5% protein).

The caseins are a second major class of milk proteins and a source of minerals and amino acids.

These proteins play a crucial role in the coagulation and curdling of milk.

This role in coagulation is also important to humans as it is a required component for cheese production [6].

K-Casein *B* allele was reported as a preferable and has significant effect on both milk and milk protein yield. Milk produced by BB genotype cows yielded significantly more cheese than that produced by *AA*-genotype cows [6].

Genotypic frequencies were 72,7 % for *AA*, 9,1% for *BB* and 18,2% for *AB*.

Frequencies of alleles *A* and *B* were 90,9 and 8,1%, respectively (table 1).

The *A* allele was more frequent than the *B* allele. Cows of *AB* and *BB* genotypes showed higher milk fat content when compared to the *AA* genotype [7].

Because of the effects of *k*-casein genetic variants on cheese yield, selection of animals with the favorable *k*- casein *B* allele is more preferable.

Prolactin *G* allele have been identified as a marker for milk quality (yield, protein and fat content) [8-10].

Genotypic frequencies were 72,7 % for *AA*, 9,1% for *GG* and 18,2% for *GA*.

Frequencies of alleles *A* and *G* were 90,9 and 8,1%, respectively (table 1).

Animals with the favorable prolactin G allele are more preferable.

The fat content is one of the most important quality characteristics of cow's milk.

It is shown that the C-allele of thyroglobulin enhances the quality of milk in terms of fat content [8].

Genotypic frequencies were 63.6% for *CC*, 8.8% for *TT* and 27.3% for *CT*.

Frequencies of alleles *C* and *T* were 90.9 and 8.1%, respectively (table 1).

Animals with the favorable thyroglobulin *T* allele are more preferable for selection.

It had been shown that growth hormone *L* allele correlated with high milk yield and fat content due to association of growth hormone with these characteristics [10].

Genotypic frequencies were 54.5% for VV, 8.9% for *LL* and 36.6% for *VL*.

Frequencies of alleles *V* and *L* were 90.9 and 8.1%, respectively (table 1). The *V* allele was more frequent than the *L* allele.

Animals with the favorable growth hormone *L* allele are more preferable and recommended for selection.

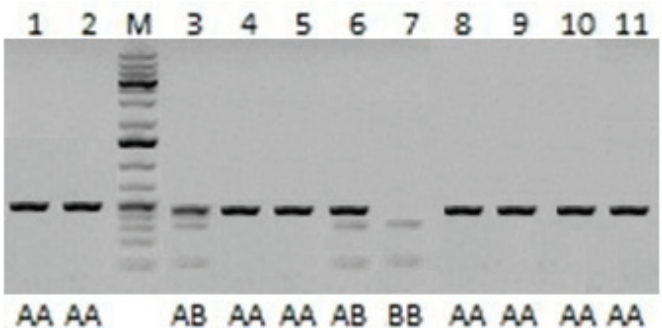

Figure 1 – Analysis of kCS amplified products; M – molecular marker, 1-11 – samples

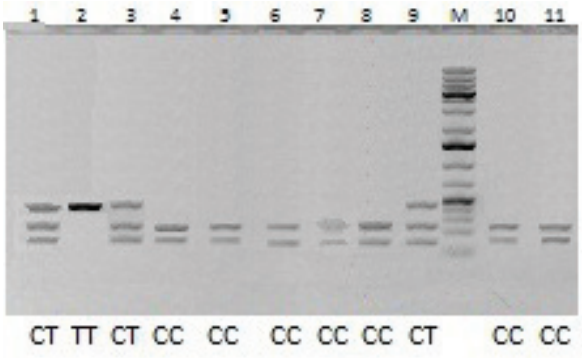

Figure 2 – Analysis of TG5 amplified products; M – molecular marker, 1-11 – samples

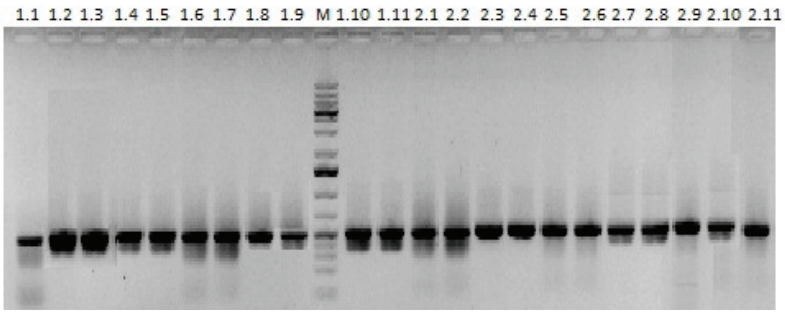

Figure 3 – M – molecular marker, 1.1-1.11 – kappa-casein amplified products (453 bp), 2.1-2.11 – thyroglobulin amplified products (473 bp)

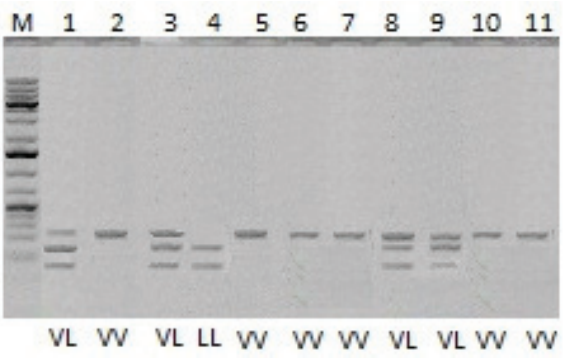

Figure 4 – Analysis of bGH amplified products; M – molecular marker, 1-11 – samples

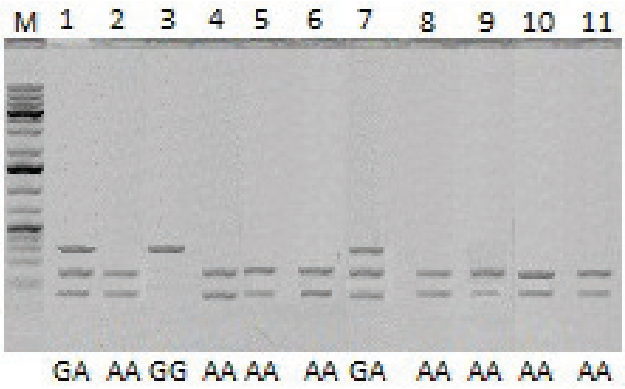

Figure 5 – Analysis of PL amplified products; M – molecular marker, 1-11 – samples

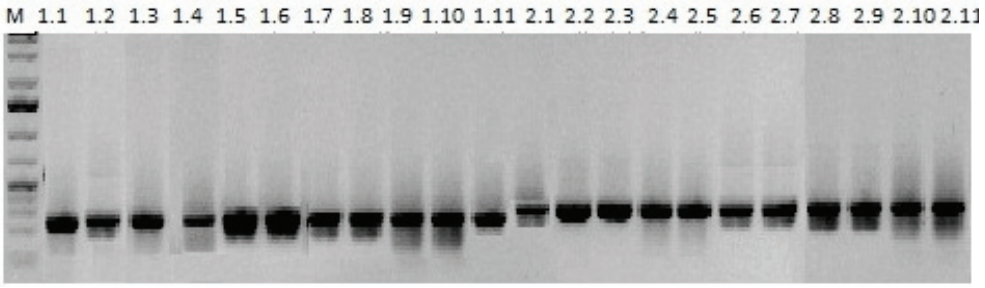

Figure 6 – M – molecular marker, 1.1-1.11 – growth hormone amplified products (208 bp), 2.1-2.11 – prolactin amplified products (294 bp)

Table 1 – The number and frequency of genotypes and alleles

Gene	Genotype	Number of sires	Genotype's frequency (%)	Allele	Allele's frequency %
bGH AluI polymorphism	*VV*	6	54,5	V	90,9
	VL	4	36,6	L	8,1
	LL	1	8,9		
TG5 BstXI polymorphism	*CC*	7	63,6	C	90,9
	CT	3	27,3	T	8,1
	TT	1	8,8		
kCS HindIII polymorphism	*AA*	8	72,7	A	90,9
	AB	2	18,2	B	8,1
	BB	1	9,1		
PL RsaI polymorphism	*AA*	8	72,7	A	90,9
	GA	2	18,2	G	8,1
	GG	1	9,1		

Thus, it is shown that among the studied animals frequency of desirable genotypes and preferable alleles is extremely low. Future studies should concentrate on the association between cattle genotype and milk composition characteristics. I addition, further research should be done on a larger scale in order to create high quality and productive herds of animals with desirable genotypes.

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