BIOLOGIJA. 2006. Nr. 1. P. 24–29

Association of cattle genetic markers with performance traits

I. Miceikienė,

N. Pečiulaitienė,

L. Baltrėnaitė,

R. Skinkytė,

R. Indriulytė

K. Janušauskas Laboratory of Animal Genetics, Department of Animal Breeding and Genetics, Lithuanian Veterinary Academy, Tilžės 18, Kaunas LT-47181, Lithuania, E-mail: genetikalab@lva.lt

Milk protein polymorphism genetic variants of bovine milk proteins Alfa$_{s1}$-CN-casein, Kapa-CN-casein, Beta-LG-lactoglobulin and polypeptide hormone prolactin (PRL) were studied in two native cattle breeds – Lithuanian Light Grey and Lithuanian White Backed and in two modern cattle breeds – Lithuanian Black and White and Lithuanian Red – by polymerase chain reaction (PCR).

The aim of this study was to investigate the distribution of cattle genetic markers and to evaluate them as potential markers of milk performance traits in Lithuanian dairy cattle breeds.

Two different alleles of polypeptidic hormone PRL were detected in four Lithuanian cattle breeds. The PRL gene A allele was found with a higher frequency in Lithuanian White Backed cattle breed and it was the least frequent in Lithuanian Red cattle breed. Three genotypes were identified for polypeptidic hormone PRL (AA, AB and BB) in Lithuanian breeds. Prolactin gene AA genotype was the most frequent in all investigated breeds. A higher influence of PRL gene was evaluated for milk fat percentage (12.12%, $p < 0.001$).

Lithuanian dairy cattle breeds were investigated for three milk protein systems and eight 8 different milk protein types. According to research results, the B types of Alpha$_{s1}$-casein were found as predominant in all four breeds studied. The most common A and B types of milk protein Kapa-CN were found at a high frequency in all cattle breeds. In Lithuanian Red, important B types were found at a higher frequency. The obtained results for three milk protein systems loci showed higher influence of Kapa-casein gene on milk protein percentage (5.9%, $P < 0.001$)

All Lithuanian dairy cattle breeds showed a high frequency of whey protein Beta-lactoglobulin B types, the highest being in Lithuanian Red breed and the lowest in Lithuanian Light Grey.

Key words: prolactin, casein, lactoglobulin, milk, cattle

INTRODUCTION

At lot of genes and quantitative trait loci have been found to influence milk yield, fat and protein content, protein quality (casein, lactoglobulin, lactoalbumin), growth hormone, prolactin and other genes. Prolactin gene (PRL) is 10 kb in size consisting of 5 egzones and 4 introns found in cattle 23 chromosome [1]. The PRL gene due to it's polymorphism (A-G mutation) at the third egzone of 103 amino acid, which creates a restriction site for RsaI, may serve as an informative molecular marker for milk yield and milk composition [2, 3]. 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 quantitative trait locus and genetic marker of production traits in dairy cattle [4].

Genes associated with milk production were investigated in many breeds to trace differences among breeds or groups of breeds [5–9]. Differences were found on the background of gene polymorphism.

In bovine milk, six major milk protein fractions (Alfa$_{s1}$-CN, Alfa$_{s2}$-CN, Kapa-CN, Beta-CN, Beta-LG and Alfa-LA) exist in different allelic forms, which are controlled by codominant autosomal genes according to the Mendelian inheritance [10]. Different variants and genetic variability of milk proteins have a significant effect on the physical and chemical properties of milk [11]. Relationships between milk protein polymorphism, production traits, composition of milk and milk manufacturing properties have been investigated and descri-

bed in several studies [12, 13]. The genetic polymorphism of milk proteins could serve as a criterion of selection and an informative marker in research.

The aims of this study were to investigate the distribution of cattle genetic markers and to evaluate them as potential markers of milk performance traits in Lithuanian dairy cattle breeds.

MATERIALS AND METHODS

Sampled breeds. The polypeptidic hormone PRL gene was investigated in 91 unrelated cattle (71 cows and 20 bulls) who represented four Lithuanian cattle breeds – 11 Lithuanian Light Grey, 36 Lithuanian Black and White, 18 Lithuanian White Back and 26 Lithuanian Red. DNA was extracted from hair roots according to the protocol obtained from Van Haeringen Laboratorium, Holland (personal contacts).

In the study of milk protein genes polymorphism, 396 animals from four Lithuanian cattle breeds were included. Blood samples were collected from 70 Lithuanian Light Grey, 49 Lithuanian White Back, 109 Lithuanian Black and White and 168 Lithuanian Red unrelated animals. DNA was extracted from blood using the standard phenol-chloroform purification method [14].

The genetic variants of PRL and milk protein have been identified by the polymerase chain reaction (PCR) methodology [15] and by restriction fragment length polymorphism (RLFP) [16].

After amplification, the 156 bp-long DNA fragment of PRL gene was digested with RsaI restriction nuclease (MBI Fermentas, Lithuania; 10 units/20 ml, at 37 °C overnight). The amplified 247 bp-long DNA fragment of whey protein Beta-LG gene was digested with Hae III restriction nuclease (MBI Fermentas, Lithuania; concentration 10 units/20 ml, at 37 °C). The amplified 337 bp-long DNA fragment of milk protein KapaCN gene was digested with Hae III and HinfI restriction nucleases (MBI Fermentas, Lithuania; 10 units/20 ml, at 37 °C).

After restriction, PCR products of PRL, Beta-LG and KapaCN were separated electrophoretically using 3% and 4% agarose gels for 35 min at 100 V. The PCR product of $Alfa_{s1}$-CN was carried out electrophoretically using 2% agarose gel.

Visualization PCR products and digested fragments were carried out after staining the gels with ethidium bromide using a Bio Doc 1000 video documentation system (BioRad, USA).

Statistical analysis. Data for this study were prepared and used by the Access database management system. The frequencies of different milk protein and PRL variants and genotypes were calculated using the POP100GENE computer program (available at: http://www.ensam.inra.fr/URLB/pop100gene/pop100gene.html).

The R statistical package was used to estimate the effects of PRL and milk protein genes on milk production traits [17].

Effects of genes PRL, Kapa-CN, $Alpha_{s1}$-CN and Beta-LG on milk yield and milk composition traits were considered by linear models.

The data on the productivity of animals were obtained from the record processing center "Kaimo verslo plėtros ir informacijos centras".

RESULTS

Two different alleles of the polypeptidic hormone PRL were detected in four Lithuanian cattle breeds. The PRL gene A allele was most frequent (0.97) in Lithuanian White Back cattle breed and the least frequent (0.77) in Lithuanian Red cattle breed. A allele frequency was very high – 0.87 in all population, whereas that of B allele was only 0.13. 87% individuals of the population had A allele and 13% had B allele. The frequencies of PRL alleles in Lithuanian cattle breeds are shown in Table 1.

Three genotypes were identified for the polypeptidic hormone PRL (AA, AB and BB) in Lithuanian cattle breeds. The prolactin gene AA genotype was the most frequent in all investigated breeds (0.62–0.94), followed by AB (0.06–0.37). The BB genotype was the least frequent (0.00–0.08). The BB genotype was not found in Lithuanian White Back and Lithuanian Light Grey cattle breeds. 75% of all investigated population had AA genotype, 18% had AB genotype and 7% BB genotype (Table 1).

Table 1. **Prolactin gene alleles and genotype frequencies in four Lithuanian cattle breeds**

Breed / Alleles	Lithuanian White Backed	Lithuanian Light Grey	Lithuanian Red	Lithuanian Black and White	In all population
A	0.97	0.95	0.77	0.80	0.87
B	0.03	0.05	0.23	0.20	0.13
Breed / Genotypes	**Lithuanian White Backed**	**Lithuanian Light Grey**	**Lithuanian Red**	**Lithuanian Black and White**	**In all population**
AA	0.94	0.91	0.62	0.71	0.75
AB	0.06	0.09	0.37	0.21	0.18
BB	0.00	0.00	0.01	0.08	0.07

Table 2. **Frequency of different milk protein types for Alpha$_{s1}$-CN, Kapa-CN and Beta-LG in four Lithuanian cattle breeds**

Milk protein types population	Breeds				
	Lithuanian dairy cattle n = 396	Lithuanian White Backed n = 49	Lithuanian Light Grey n = 70	Lithuanian Red n = 168	Lithuanian Black and White n = 109
Alpha$_{s1}$-CN					
B	0.949	0.948	0.976	0.985	0.885
C	0.050	0.051	0.024	0.015	0.115
Kapa-CN					
A	0.739	0.731	0.735	0.714	0.752
B	0.218	0.238	0.228	0.265	0.161
E	0.041	0.010	0.037	0.021	0.087
Beta-LG					
A	0.290	0.378	0.551	0.068	0.431
B	0.705	0.622	0.449	0.923	0.569
C	0.003	–	–	0.009	–

Table 3. **Alpha$_{s1}$-CN, Kapa-CN and Beta-LG genotype frequencies in Lithuanian dairy cattle breeds**

Milk protein types population	Breeds				
	Lithuanian dairy cattle n = 396	Lithuanian White Backed n = 49	Lithuanian Light Grey n = 70	Lithuanian Red n = 168	Lithuanian Black and White n = 109
Alpha$_{s1}$- CN					
BB	0.903	0.932	0.968	0.979	0.775
BC	0.091	0.068	0.032	0.021	0.224
CC	0.005	–	–	0.012	–
Kapa-CN					
AA	0.525	0.477	0.492	0.553	0.561
AB	0.363	0.364	0.429	0.369	0.265
AE	0.066	0.045	0.032	0.028	0.122
BB	0.018	0.114	0.032	0.042	–
BE	0.028	–	0.016	0.007	0.051
Beta-LG					
AA	0.132	0.136	0.206	0.007	0.163
AB	0.317	0.477	0.508	0.128	0.469
BB	0.543	0.387	0.286	0.851	0.367
BC	0.007	–	–	0.014	–

Lithuanian dairy cattle breeds were investigated for three milk protein systems, eight different milk protein types. The frequencies of Alpha$_{s1}$-CN, Kapa-CN, and Beta-LG types for each breed separately were estimated.

According to the research results, the B types of Alpha$_{s1}$-CN were found as predominant in all four breeds studied and varied from 0.885 for Lithuanian Black and White to 0.985 for Lithuanian Red. The C types of Alfa$_{s1}$-CN, which may confer a higher protein level in milk, was most common in Lithuanian Black and White breed (0.115), while in other populations it appeared at a very low frequency (Table 2). The milk protein Alpha$_{s1}$-CN BB genotype had a positive influence on milk yield; it was most common to the dairy cattle and was observed at high frequency in all four Lithuanian cattle breeds studied. However, the BB genotype of Alpha$_{s1}$-CN was highly frequent in Lithuanian Red breed (0.979), and the CC genotype was found only in Lithuanian Red (Table 3).

The most common A and B types of milk protein Kapa-CN were found at a high frequency in all four cattle breeds. In Lithuanian dairy cattle breeds, important B types were found at frequencies ranging from 0.265 for Lithuanian Red to 0.161 for Lithua-

Table 4. **Influence of genetic factors on milk yield and composition in Lithuanian dairy cattle breeds**

Genetic and Non- genetic factors	Number of classes	Milk, kg	Fat, %	Fat, kg	Protein, %	Protein, kg
PRL	3	4.98%**	12.12%***	5.26%**	6.49%***	6.77%***
Kapa-CN	5	2.5%**	3.8%***	2.17%***	5.9%***	0.90%***
Beta-LG	4	1.6%*	2.5%*	1.17%**	3.7%	0.95%
$Alpha_{s1}$-CN	3	1.0%*	0.50%*	0.60%*	0.50%***	0.65%***
Breed	4	13.6 %*	5.3%***	13.2%**	7.7%	10.3%
Herd	80	15.8%***	22.6%***	19.3%***	9.5%***	15.7%***
Lactation	8	8.5 %***	0.5%	6.8%	3.3%***	10.4%***
Regression with milk amount	c	–	0.01%	45.0%***	0.04%	52.2%***

*P < 0.05; ** P < 0.01; *** P < 0.001.

nian Black and White. According to B types of Kapa-CN selection of animals and creation of specific cattle herds are carried out in some countries. Rare E types of Kapa-CN were identified in all Lithuanian dairy cattle breeds at a very low frequency. E types of Kapa-CN were detected at a highest frequency (0.087) only in Lithuanian Black and White. The detection of E allele in all studied Lithuanian breeds reflected that these breeds might belong to Lowland breeds or might be improved by using Lowland cattle breeds (Table 2). The AA and AB genotypes of Kapa-CN were detected in all dairy breeds studied at a relatively high frequency. In Lithuanian White Backed, Lithuanian Light Grey and Lithuanian Red breeds the favorable BB genotype was detected at a very low frequency and was totally absent in the Lithuanian Black and White breed. A higher frequency of Kapa-CN BB genotype has been detected in Lithuanian White Backed cattle (0.114) (Table 3).

All Lithuanian dairy cattle breeds had a high frequency of Beta-LG B types. The highest frequency was found in Lithuanian Red breed (0.923) and the lowest in Lithuanian Light Grey (0.449) (Table 2). The milk with the BB genotype of Beta-LG contained more casein and fat than the milk produced by the Beta-LG AA genotype. The BB genotype of Beta-LG was observed at a very high frequency in Lithuanian Red breed (0.851), and the BC genotype was found with a low frequency (0.014) only in Lithuanian Red breed. The AA genotype of Beta-LG, which has an effect on milk and protein yield, was detected in all breeds studied with a frequency ranging from 0.007 Lithuanian Red to 0.206 Lithuanian Light Grey breeds (Table. 3).

Multifactor dispersive analysis (ANOVA) data were calculated for effects of polypeptid hormone PRL, milk protein Kapa-CN, $Alpha_{s1}$-CN and Beta-LG genes on milk production traits. This analysis (ANOVA) showed an individual influence of PRL and milk protein $Alpha_{s1}$-CN, Kapa-CN, and Beta-LG genes on milk yield and milk composition. Multifactor dispersive analysis (ANOVA) according to all genetic factors showed a higher influence of PRL gene on milk fat percentage (12.12%, P < 0.001), whereas, for three milk protein systems the ANOVA analysis showed a higher influence of Kapa-casein gene on milk protein percentage (5.9%, P < 0.001) (Table 4).

DISCUSSION

In Marker-Assisted Selection of dairy cattle, some genes are proposed as potential candidates associated with dairy performance traits. Genes influencing milk production have been investigated in a lot of breeds in order to find interbreed and intrabreed polymorphism and association with production traits. For the first time polypeptidic hormone PRL gene polymorphism was investigated in Lithuanian cattle breeds. The results correlate with results reported by other researchers. A high frequency of PRL gene A allele (0.95) was found in Holstein by several authors [16, 17]. At a similar frequency (0.87) this allele was found in Poland Red, Bohemia Red (0.62) [26], Russian Ayrshire (0.86), Gorbatov Red (0.92) [16], Argentina Holstein (0.87), Argentina (Kreole) 0.96 [18].

The frequencies of PRL genotypes obtained in this study are similar to those reported in [19] for the Polish Red and Polish Black and White cattle.

The milk protein $Alfa_{s1}$-CN B type is known as best presented in cattle and can be found with a frequency of 90–95%. In our study, this type was predominant in all breeds studied. According to Ng-Kwai-Hang [13], $Alfa_{s1}$-CN B types are associated with a high milk yield. The C types of $Alfa_{s1}$-CN are

associated with higher protein levels in milk. A surprisingly high frequency C types was found in Swedish Holstein [20]. The BB genotype of $Alfa_{s1}$-CN has a positive influence on milk yield, it is most common to dairy cattle [21] and was observed at the highest frequency in all four Lithuanian cattle breeds studied.

Desirable B types of Kapa-CN, related to the higher protein percentage and renneting time had a favorable effect on the concentration of milk components, as well as physico-chemical and technological properties of milk [22, 23]. In Lithuanian dairy cattle breeds, important B types were found at higher frequencies. Rare E types of Kapa-CN, originated from Lowland cattle breeds such as Ayrshire, Holstein, Germany Red and White [10], were identified in all Lithuanian dairy cattle breeds at a very low frequency. The frequency of AB genotypes of Kapa-casein were relatively high in all dairy breeds. In Lithuanian White Backed, Lithuanian Light Grey and Lithuanian Red breeds the favorable BB genotype was detected at a very low frequency and was totally absent in Lithuanian Black and White breed. According to Bonvillani [24], the Kapa-casein BB genotype was absent in Argentine Holstein cattle. A higher frequency of Kapa–casein BB genotype has been detected in Lithuanian White Backed cattle.

Whey protein Beta-LG of only A and B types was spread in many European dairy cattle breeds [25]. Favourable for milk industry B types of Beta-LG were most common to European cattle breeds like Ayrshire, Shorthorn and Red Danish and had been known as predominant in the Holstein breed. The BB genotype of Beta-LG was highly frequent in Lithuanian Red breed, and the BC genotype was found at a low frequency only in Lithuanian Red breed. The AA genotype of Beta-LG, which has an effect on milk and protein yield, was detected in all studied breeds, however, a higher frequency was definited in Lithuanian Light Grey breed.

The ANOVA analysis showed an individual influence of polypeptidic hormone PRL and milk protein $Alpha_{s1}$-CN, Kapa-CN, and Beta-LG genes on milk yield and composition. A higher influence of PRL gene was evaluated for milk fat percentage. The results are in accordance with the data reported by Dypus [1].

The obtained results for three milk protein system loci showed a higher influence of Kapa-casein gene on milk protein percentage, whereas most statistically significant influence was shown by whey protein Beta-lactoglobulin gene on fat percentage. Our obtained results coincide with literature data [11, 23, 24].

The usage of genetic markers allows to evaluate an animal and employ the useful traits of this animal in selection of agricultural livestock. Genetic markers can be used for identification of both a single gene and a gene group, of a definite feature or a group of features. Another advantage of genetic markers is that this animal evaluation method is reliable, economically undemanding and allows to detect the genes the control selection and technological breeding values in the young age. Using the genetic markers in selection, we can expedite the process of selection, improve the quality of agricultural production, decrease production cost and make the production competitive in foreign markets.

Received 5 December 2005
Accepted 18 February 2006

References

1. Dybus A. Animal Sci 2002; 4: 203–12.
2. Collier RJ, McNamara JP, Wallace CR, Dehoff MH. Animal Sci 1984; 59: 495–510.
3. Tucker HA. Dairy Sci 1981; 4: 1403–21.
4. BRYM P, Kaminski S, Wojcik E. Animal Genetics 2005; 45(2): 179–85.
5. Aschaffenburg R, Thymann M. Dairy Science 1965; 48: 1524–6.
6. Baker CMA., Manwell C. Anim Blood Groups Biochem Genet 1980; 11: 127–50.
7. Medjugorac I, Kustermann W, Lazar P, Russ I, Pirchner F. Animal Genetics 1994; 25: 19–27.
8. Poli MA, Antonini AG. Heredity 1992; 115: 177–82.
9. Velmala R, Mysaari EA, Tanila A. Agric Scie Finl 1993; 2: 431–5.
10. Erhardt G, Godovac-Zimmermann J, Juszczak J, Prinzenberg EM, Krick-Saleck H, Panicke L. Proc of the 48th EAAP Meeting, 25–28 August. Vienna, Austria 1997: 1–7.
11. Shaar J, Hansson B, Pettersson HE. Dairy Research 1985; 52: 429.
12. Lien S, Alestrom P, Klungland H, Rogne S. Animal Genetics 1992; 23: 295–300.
13. Ng-Kwai-Hang KF, Grosclaude F. Dairy Chem 1992; 1: 405–55.
14. Miller SA, Dykes D, Polecky HE. Nucleic Acids Res 1988; 16: 3.
15. Sakai RK, Gelfand DH, Stoffel S, Scharf SJ, Higuchi R, Horn GT, Mullis KB, Erlich HA. Animal Sci 1988; 239: 487–91.
16. Chrenek P, Vasicek D, Bauerova M, Bulla J. Czech J Animal Sci 1998; 43: 53–5.
17. Chung ER, Rhim TJ, Han SK. Korean J Animal Sci1996; 38: 321–36.
18. Ng-Kuai-Hang KF, Hayes JF, Moxley JE, Morandes HG. J Dairy Sci 1984; 67: 835–42.
19. Klauzinska M, Klewiec J, Zurkowski M, Siadkowska E, Szymanowska M, Grochowska R, Zwierzchowski L. Animal Sci 2004; 22(1): 45–56.
20. Lunden A, Nilsson M, Janson L. J Dairy Sci 1997; 80: 2996–3005.
21. Aleandri R, Butazzoni G, Scneider JC, Caroli A, Davoli R. Dairy Sci 1990; 73: 241–55.

22. Strzalkowska N, Krzyzewski J, Zwierzchowski L, Ryniewicz Z. Animal Science. 2002; 1: 21–35.
23. Van Eenennaam A, Medrano JF. Dairy Sci 1991; 5: 74.
24. Bonvillani AG, Di Renzo MA, Tiranti IN. Genet Mol Biol Sao Paulo 2000; 4: 23.
25. Hill JP, Boland MJ, Creamer LK. Livestock Production Sci 1993; 35: 193.
26. Kopenčy M, Nebola M, Dvorak J. J Central Europ Agricult 2004; 4: 303–8.

I. Miceikienė, N. Pečiulaitienė L. Baltrėnaitė, R. Skinkytė, R. Indriulytė

GALVIJŲ GENETINIŲ ŽYMENŲ RYŠYS SU PRODUKTYVUMO SAVYBĖMIS

S a n t r a u k a

Galvijų pieno baltymų alfa$_{s1}$ kazeino, kappa kazeino, beta laktoglobulino ir polipeptidinio hormono prolaktino (PRL) genetinių tipų polimorfizmui nustatyti buvo paimtos dvi vietinės galvijų veislės – Lietuvos šėmieji ir Lietuvos baltnugariai bei dvi modernios galvijų veislės – Lietuvos juodmargiai ir Lietuvos žalieji. Genai identifikuoti PGR-RFIP metodu.

PRL nustatytos dvi skirtingos alelės keturiose Lietuvos galvijų veislėse. PRL geno A alelis aukščiausiu dažniu aptiktas Lietuvos baltnugarių veislėje, o mažiausiu – Lietuvos žalųjų galvijų veislėje. Analizuojamose veislėse identifikuoti trys PRL genotipai (AA, AB ir BB). PRL geno AA genotipas atitinkamai aukštu dažniu nustatytas visose tirtose veislėse. Statistiškai patikimas PRL geno poveikis gautas pieno riebalų procentui 12,12% ($p < 0,001$).

Analizuojamose veislėse buvo tirti trijų pieno baltymų sistemų aštuoni skirtingi pieno baltymų tipai. Tyrimais nustatyta, kad pieno baltymo alfa$_{s1}$ kazeino B alelis vyravo visose analizuojamose pieninių galvijų veislėse. Daugelyje pasaulio galvijų veislių dažniausiai randamas kapa kazeino A alelis buvo rastas aukštu dažniu visose tirtose veislėse. Tuo tarpu pieno baltymo kapa kazeino B alelis didžiausiu dažniu buvo nustatytas Lietuvos žalųjų galvijų veislėje. Mūsų studijų duomenimis, didžiausias kapa kazeino geno poveikis nustatyta pieno baltymų procentui: kapa kazeino genas veikė 5,9% pieno baltymų ($p < 0,001$). Išrūgų baltymo beta laktoglobulino B alelis aukščiausiu dažniu rastas Lietuvos žalųjų galvijų veislėje, žemiausiu dažniu – Lietuvos šėmųjų galvijų veislėje.