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Genetic factors influencing milk production traits in Lithuanian dairy cattle breeds

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394 unrelated Lithuanian dairy cows (Lithuanian Black & White 109, Lithuanian Red 168, Lithuanian Light Grey 68 and Lithuanian White Backed 49) were evaluated to test the influence of milk protein genotypes milk to production and milk composition traits. Milk protein genes were identified by polymerase chain reaction (PCR) and the RFLP method. The results showed a positive effect of the BB genotype of kappa-casein on milk fat and protein content, however, this genotype had a negative effect on milk yield. The Alpha$_{s1}$-casein BB genotype showed milk yield, whereas the alpha$_{s1}$-casein CC genotype produced more protein. The whey protein BB genotype of the beta-lactoglobulin locus influenced milk fat level, whereas the beta-lactoglobulin BC genotype was produced more protein. Differences among the milk protein genotypes were estimated. Cows with kappa-casein locus AA genotype produced more milk (175.7 kg; $p < 0.05$) and a higher (2.88 kg; $P < 0.001$) protein average than did AB genotype carrier cows. However, milk of cows with AB genotype was superior according to fat (0.23%; $p < 0.001$) and protein (0.06%; $p < 0.001$) content. Milk of cows with the kappa-casein locus BB genotype, beneficial for milk processing, was superior according to fat (0.27%; $p < 0.05$) and protein (0.21%; $p < 0.001$) average levels versus the kappa-casein locus AA genotype. Comparing the differences between the BB and CC genotypes of alpha$_{s1}$-casein it was estimated that cows of the BB genotype produced more milk (1344 kg; $p < 0.05$), however, in cows of the CC genotype milk had a higher (by 0.35%) protein. Cows carrying the AB genotype of beta-lactoglobulin produced more milk (by 332.2 kg; $p < 0.01$) and milk had more (by 0.10%, $p < 0.05$) fat compared with cows that carried AA genotype. The BB genotype influenced milk protein level (by 0.20%, $p < 0.001$) more than did the AA genotype.

According to the research results, in respect of beta-lactoglobulin and kappa-casein genotypes of milk protein, it could be possible to certify pedigree cattle – to select cows, bulls and their semen genetically, testing genes determining the qualitative composition of milk protein. The results may be used to improve genetically their breeding value and the quality of milk.

Key words: casein, lactoglobulin, milk, cattle

INTRODUCTION

The geographical situation, climate and feed supply are very favourable for dairy cattle breeding development in Lithuania. Dairy cattle breeding is taking priority in agriculture and food industry. Biologically, milk is a very valuable food product available from dairy cattle. Milk and its products are significant for human nutrition as they contain all substances necessary for human organism, such as fat, protein, carbohydrates and minerals. Milk contains over 200 various substances essential for cell resumption and energy supply in human organism

[8]. Milk as a food product passes a long and tricky way from newborn food to a wide assortment of milk products [4]. Nowadays Lithuanian food industry has a technological potential to produce various dairy products of more than 130 names. Nutrition specialists from different countries recommend to consume 400–500 kg of dairy products per year: 180 kg of raw milk, 135 kg of butter, 185 kg of cheese, curd, sour cream and other dairy products per person [9].

Milk yield and its constituent substances are classical quantitative traits influenced by various genes and environmental conditions [9]. The processing properties of milk are related to the composition of its proteins [1]. As the types of caseins and lactoglobulins are controlled by autosomal genes, it may be important to verify the existence of genotypes that give better results in terms of cheese production [3]. Different variants and genetic variability of milk proteins have a significant effect on its physical and chemical properties [14]. It has been reported that specific genetic variants of milk proteins, especially of caseins, are of significant importance in cheese making as cheese yield is related to the casein composition and casein content in milk [6].

The aim of this study was to investigate the influence of milk protein genetic variants on the yield and milk constituent components in Lithuanian dairy cattle breeds.

MATERIALS AND METHODS

394 unrelated Lithuanian dairy cows: Lithuanian Black & White – 109, Lithuanian Red – 168, Lithuanian Light Grey – 68 and Lithuanian White Backed – 49 were evaluated to test the influence of milk protein genotypes on the productivity of milk and milk composition traits.

Data on the productivity of animals were obtained from the Record Processing Center SE "Kaimo verslo plėtros ir informacijos centras".

Blood samples for DNA genotyping were extracted using a standard phenol–chloroform purification method [10]. The milk protein genotypes were tested by a methodology based on polymerase chain reaction (PCR) [13]. The amplified DNA fragments were digested with restriction nucleases (RFLP) [15].

Casein genotype analysis. For PCR reaction alpha$_{s1}$-casein primers were used: A primer 5'-GGC ACA CAA TAC ACT GAT GC-3'; B primer 5'-CAG TGG CAT AGT AGT CTT TT-3' and C primer 5'-CAG TGG CAT AGT AGT CTT TC-3'. DNA was amplified in 34 cycles (94 °C 30 s, 60 °C 30 s, 72 °C 30 s) followed by 5 min at 72 °C by Geneamp PCR system 2700. The PCR product was treated electrophoretically using 2% agarose gel.

Beta-lactoglobulin genotype analysis. For PCR reaction beta-lactoglobulin primers were used: JBLG 2-5'-TGT GCT GGA CAC CGA CTA CAA AAA G-3' and JBLG 3-5'-GCT CCC GGT ATA TGA CCA CCC TCT-3'. DNA was amplified in 35 cycles (94 °C 40 s, 58 °C 50 s, 72 °C 50 s) followed for 5 min at 72 °C by Geneamp PCR system 2700. The amplified 247 bp-long DNA fragment was digested with *Hae III* restriction nuclease (MBI Fermentas, Lithuania; concentration 10 units / 20ml, at 37 °C). After restriction the products were separated electrophoretically using 3% agarose gel for 35 min at 100 V.

Kappa-casein genotype analysis. For PCR reaction kappa-casein primers were used: K346A-5'-CAT-TTA-TGG-CCA-TTC-CAC-CAA-AG-3' and K346B-5'-CAT-TTC-GCC-TTC-TCT-GTA-ACA-G-3'. DNA was amplified in 34 cycles (94 °C 30 s, 58 °C 30 s, 72 °C 30 s) followed for 5

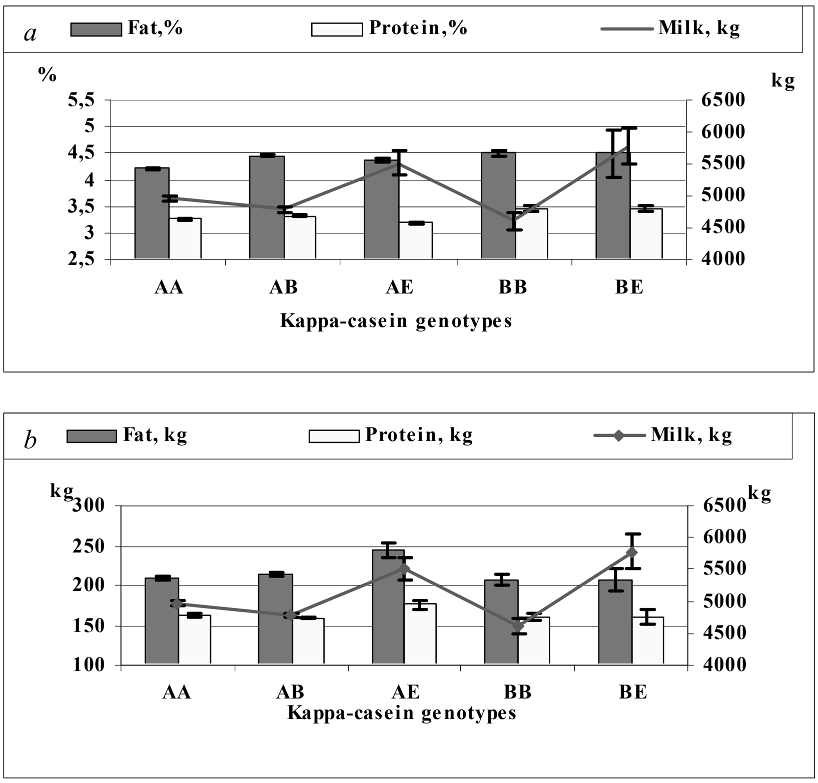

Fig. 1. Milk protein kappa-casein genotypes according to milk yield and milk constituents in Lithuanian dairy cattle breeds: *a* – fat %, protein %, milk kg, *b* – fat kg, protein kg, milk kg

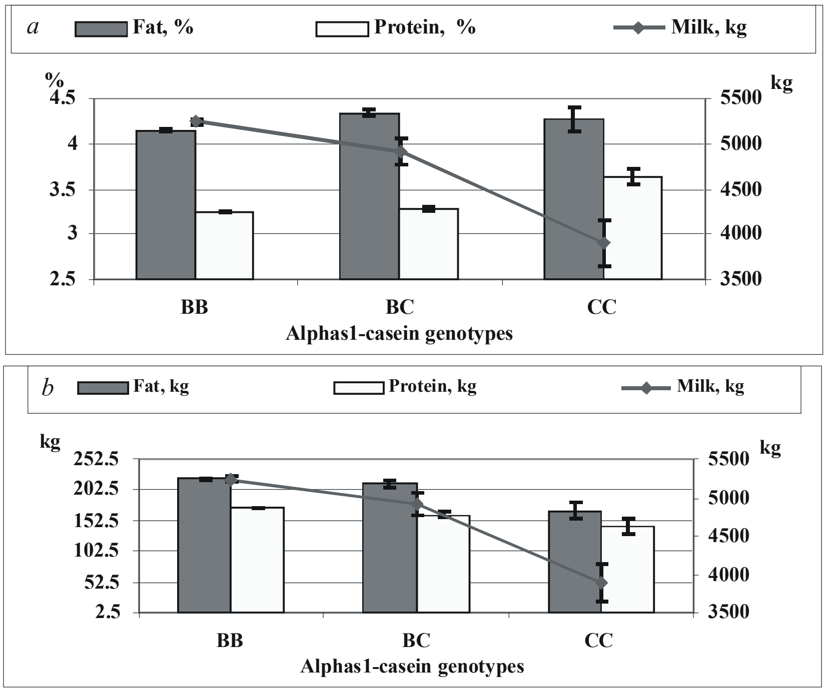

Fig. 2. Milk protein alpha$_{s1}$-casein genotypes according to milk yield and milk constituents in Lithuanian dairy cattle breeds: *a* – fat, %, protein, %, milk, kg, *b* – fat, kg, protein, kg, milk, kg

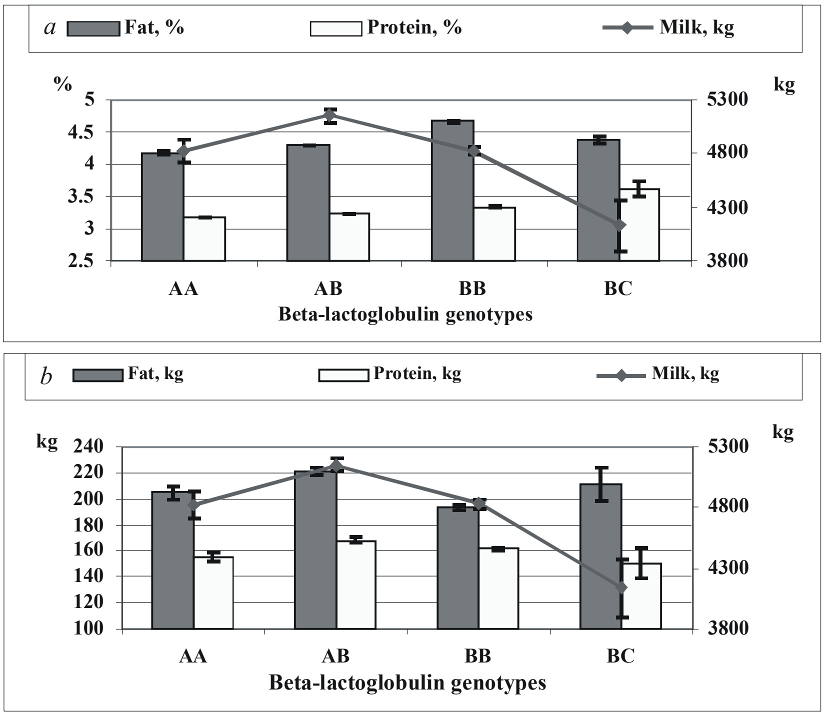

Fig. 3. Whey protein beta-lactoglobulin genotypes according to milk yield and milk constituents in Lithuanian dairy cattle breeds: *a* – fat, %, protein, %, milk, kg, *b* – fat, kg, protein, kg, milk, kg

min at 72 °C by Geneamp PCR system 2700. The amplified 337 bp-long DNA fragment was digested with *Hae III* and *HinfI* restriction nucleases (MBI Fermentas, Lithuania; 10 units / 20 ml, at 37 °C). After restriction the products were separated electrophoretically using 3% agarose gel, 35 min at 100 V.

Visualization of the different milk protein genetic variants was carried out after staining the gels with ethidium bromide, using the Bio Doc 1000 video documentation system (BioRad, USA).

Statistical analysis. Data for this study were prepared and used by the Access database management system. Effects of milk protein kappa-casein, alpha$_{s1}$-casein and beta-lactoglobulin genotypes on milk production traits were estimated using the R statistical package [5].

RESULTS AND DISCUSSION

The parameters of milk yield and milk composition were estimated according to all milk protein genotypes found in the Lithuanian dairy cattle population. It was estimated that the BB genotype of milk protein kappa-casein locus affected major milk fat (4.50 ± 0.5%) and protein (3.47 ± 0.04%) averages, whereas the kappa-casein BE genotype could be characterized by a higher milk yield average (5776 ± 27 kg). The obtained results showed that the BB genotype exerts a positive effect on milk protein composition; however, this genotype has a negative effect on milk yield (Fig. 1). Our results coincided with literature data [2, 7].

Alpha$_{s1}$-casein BB genotype effected higher milk yield average (5242 ± 14 kg), whereas alpha$_{s1}$-casein CC genotype was superior in protein average (3.64 ± 0.09%) (Fig. 2). According to literature [11, 12], the alpha$_{s1}$-casein BB genotype affectes higher milk yield than do the BC and CC genotypes.

The whey protein BB genotype of beta-lactoglobulin locus influenced major milk fat (4.67 ± 0.01%) average, whereas the beta-lactoglobulin BC genotype was superior in protein average (3.6 ± 0.12%) (Fig. 3). Whey protein beta-lactoglobulin influenced milk fat percentage and casein stock in milk [11, 12, 16].

Differences among milk protein kappa-casein genotypes were estimated. Cows with kappa-casein locus AA genotype produced more milk (by 175.7 kg, P < 0.05) and protein (2.88 kg, P < 0.001) on average than did cows with the AB genotype carried. However, milk of cows with AB genotype was superior according to fat (0.23%, P < 0.001) and protein (0.06%, P < 0.001) average values. In cows with kappa-casein locus BB genotype, beneficial for milk processing, milk was superior according to fat (0.27%, P < 0.05) and protein (0.21%, P < 0.001) average values as compared with cows that had kappa-casein locus AA genotype. The BB genotype of kappa-casein locus plays an important role in milk properties needed for cheese and curd formation. Also, the BB genotype was superior by protein average (0.15%, P < 0.05) compared with cows with the kappa-casein locus AB genotype. The AE genotype of milk protein kapa-casein locus influenced the major milk yield (899.3 kg, P < 0.001), fat

Table 1. **Differences among milk protein kappa-casein genotypes according to milk yield and milk constituents in Lithuanian dairy cattle breeds**

Differences among milk protein kappa-casein genotypes according to milk yield, kg				
Genotype	AA	AB	AE	BB
AB	–175.7*	–	–	–
AE	–549.0*	+724.7***	–	–
BB	–350.3	–174.7	–899.3***	–
BE	+812.6*	+988.2***	+263.6	+1163***
Differences among milk protein kappa-casein genotypes according to milk fat, %				
Genotype	AA	AB	AE	BB
AB	+0.23***	–	–	–
AE	+0.13*	–0.09	–	–
BB	+0.27*	+0.04	+0.14	–
BE	+0.09	–0.13	–0.03	–0.17
Differences among milk protein kappa-casein genotypes according to milk fat, kg				
Genotype	AA	AB	AE	BB
AB	+2.79***	–	–	–
AE	+33.3*	+30.6***	–	–
BB	–3.48	–6.27	–36.8*	–
BE	+41.0	+3.2**	+7.64	+44.5*
Differences among milk protein kappa-casein genotypes according to milk protein, %				
Genotype	AA	AB	AE	BB
AB	+0.06***	–	–	–
AE	–0.06	–0.12***	–	–
BB	+0.21***	+0.15*	+0.27***	–
BE	–0.04	–0.06	+0.06	–0.21*
Differences among milk protein kappa-casein genotypes according to milk protein, kg				
Genotype	AA	AB	AE	BB
AB	–2.88**	–	–	–
AE	+13.2*	+16.7**	–	–
BB	–1.89**	+0.99	–15.1**	–
BE	+25.0*	+27.9*	+11.8	+26.9

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

Table 2. **Differences among milk protein alfa$_{s1}$-casein genotypes according to milk yield and milk constituents in Lithuanian dairy cattle breeds**

Differences among milk protein alfa$_{s1}$-casein genotypes according to milk yield, kg		
Genotype	BB	BC
BC	–328*	–
CC	–1344***	–1015*
Differences among milk protein alfa$_{s1}$-casein genotypes according to milk fat, %		
Genotype	BB	BC
BC	–0.18*	–
CC	+0.11	–0.07
Differences among milk protein alfa$_{s1}$-casein genotypes according to milk fat, kg		
Genotype	BB	BC
BC	–6.63*	–
CC	–52.0	–45.4
Differences among milk protein alfa$_{s1}$-casein genotypes according to milk protein, %		
Genotype	BB	BC
BC	+0.04	–
CC	+0.40	+0.35*
Differences among milk protein alfa$_{s1}$-casein genotypes according to milk protein, kg		
Genotype	BB	BC
BC	–11.0*	–
CC	–29.4	–18.4

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

Table 3. **Differences among whey protein beta–lactoglobulin genotypes according to milk yield and milk constituents in Lithuanian dairy cattle breeds**

Differences among whey protein beta-lactoglobulin genotypes according to milk yield, kg			
Genotype	AA	AB	BB
AB	+332.2**	–	–
BB	+9.90	–322***	–
BC	–687.6	–1020*	–697.5
Differences among whey protein beta-lactoglobulin genotypes according to milk fat, %			
Genotype	AA	AB	BB
AB	+0.10*	–	–
BB	+0.20***	+0.09	–
BC	+0.48**	+0.38*	+0.28*
Differences among whey protein beta-lactoglobulin genotypes according to milk fat, kg			
Genotype	AA	AB	BB
AB	+16.6	–	–
BB	+7.09	–9.53	–
BC	–11.9*	–28.2	–18.6*
Differences among whey protein beta-lactoglobulin genotypes according to milk protein, %			
Genotype	AA	AB	BB
AB	+0.06	–	–
BB	+0.15	+0.08	–
BC	+0.45	+0.38	+0.30
Differences among whey protein beta-lactoglobulin genotypes according to milk protein, kg			
Genotype	AA	AB	BB
AB	+12.7	–	–
BB	+6.06	–6.70	–
BC	–4.68	–17.4	–10.7

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

(36.8 kg, P < 0.05) and protein (15.1 kg, P < 0.01) average values, than did kappa-casein BB genotype. Comparison of AE genotype with AB genotype of milk protein kappa-casein locus showed that cows with AE genotype produced higher milk (724.7 kg, P < 0.001), fat 30.6 kg (P < 0.001) and protein (16.7 kg, P < 0.01) average values. The AB genotype was superior than the AE genotype (0.12%, P < 0.01) in protein average. Similarly, a statistically significant difference was estimated between the kappa-casein locus BE and AA genotypes in milk yield and protein content. Cows with BE genotype produced more yield (812.6 kg, P < 0.05) and protein (25.0 kg, P < 0.05) compared with cows with the kappa-casein locus AA genotype. Comparing the BE and AB genotype of milk protein kappa-casein locus, the BE genotype was superior by 988.2 kg (P < 0.001) in milk yield, 38.2 kg (P < 0.01) in fat and 27.9 kg (P < 0.05) in protein average values. Similarly, a statistically significant difference was obtained between the BE and the BB genotypes: cows having the BE genotype produced more milk (1163 kg, P < 0.001) and fat (44.5 kg, P < 0.05) than cows with the BB genotype (Table 1).

Statistically significant differences were found between BB and BC genotypes of the alfa$_{s1}$-casein locus. Cows of the BB genotype produced more milk (328 kg, P < 0.05), fat (6.63 kg, P < 0.05) and protein (11.0 kg, P < 0.05), whereas the milk of cows of the BC genotype was superior by 0.18% in fat. Comparison of the BB and CC genotypes showed that cows of the BB genotype produced more milk (1344 kg, P < 0.05); however, in cows of the CC genotype milk was superior (0.35%) in protein. Statistically significant differences were found between BC and CC genotypes according to milk yield average. The BC genotype of Alfa$_{s1}$-casein influenced milk yield (1015 kg, P < 0.001) (Table 2).

Cows carrying the AB genotype produced more milk (332.2 kg, P < 0.01) and their milk had more (0.10%, P < 0.05) fat compared with cows that carried the AA genotype. The BB genotype of beta-lactoglobulin induced more protein (0.20%, P < 0.001) than did the AA genotype. Analysis of differences between the AA and BC genotypes revealed that cows with the BC genotype contained more fat (P < 0.01). Statistically significant differences were detected between AB and BB genotypes for milk yield. The AB genotype cows produced more milk (322 kg, P < 0.001) than did cows carrying the BB genotype. Similarly, statistically significant differences were detected between AB and BC genotypes in whey protein beta-lactoglobulin according to milk yield in kilograms and in fat percentage. Cows of the AB genotype produced a higher milk yield (1020 kg, P < 0.05), meanwhile the milk of the BC genotype cows contained more fat (0.38%, P < 0.05). Comparison of differences between the BB and BC genotypes showed that milk of BB genotype contained more milk fat by 0.28% (P < 0.05) and kilogram 18.6 kg (P < 0.05) (Table 3).

CONCLUSION

According to the research results in respect of beta-lactoglobulin and kappa-casein genotypes of milk protein, it could be possible to certify pedigree cattle, i. e. to select cows, bulls and their semen genetically, testing genes that determine the qualitative composition of milk proteins. The obtained results could be used to improve animal breeding and to improve genetically the quality of milk. The genetic diversity of milk proteins could serve as a criterion of selection and the informative marker in research of the phylogenetic relationships and evolution of breeds.

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GENETINIŲ VEIKSNIŲ POVEIKIO PIENO PRODUKCIJOS SAVYBĖMS LIETUVOS PIENINIŲ GALVIJŲ VEISLĖSE ĮVERTINIMAS

Santrauka

Pieno baltymus lemiančių genų įtakai produktyvumui ir pieno sudėčiai nustatyti buvo ištirtos 394 negiminingos Lietuvos pieninių veislių karvės: iš jų 109 Lietuvos juodmargės, 168 Lietuvos žalosios, 68 Lietuvos šėmos ir 49 Lietuvos baltnugarės karvės. Pieno baltymų genotipai identifikuoti PGR–RFIP (polimerazės grandinės reakcijos, restrikcinių fragmentų ilgio polimorfizmo) metodu. Gauti rezultatai parodė, kad kapa kazeino BB genotipas turėjo teigiamą įtaką baltymų ir riebalų kiekiui piene, tačiau neigiamą – pieno išeigai. Pieno baltymo alfa$_{s1}$ kazeino BB genotipas turėjo įtakos didesniam pieningumui, tuo tarpu CC genotipas – didesniam pieno baltymingumui. Išrūgų baltymo beta laktoglobulino BB genotipo didžiausias poveikis nustatytas riebalams, BC genotipo – baltymų kiekiui piene. Įvertinti pieno baltymų genotipų skirtumai. Karvės su AA genotipu pasižymėjo didesniu 175,7 kg ($P < 0,05$) pieno ir 2,88 kg ($P < 0,001$) baltymų kiekiu, nei AB genotipo karvės. Tačiau AB genotipą turinčių karvių pienas buvo 0,23% ($P < 0,001$) riebesnis ir 0,06% ($P < 0,001$) baltymingesnis. Nustatyta, kad kapa kazeino lokuso BB genotipo karvių pienas vidutiniškai 0,27% ($P < 0,05$) riebesnis ir 0,21% ($P < 0,001$) baltymingesnis, lyginant su AA genotipą turinčių karvių pienu. Lyginant genotipų skirtumus tarp alfa$_{s1}$ kazeino lokuso BB genotipo ir CC genotipo karvių, buvo gauta, kad BB genotipo karvės davė 1344 kg ($P < 0,05$) daugiau pieno, tačiau CC genotipo karvių pienas buvo 0,35% baltymingesnis. Įvertinus skirtumus tarp pieno baltymo beta laktoglobulino lokuso genotipų, buvo nustatyta, kad AB genotipą turinčios karvės davė vidutiniškai 332,2 kg ($P<0,01$) daugiau pieno ir pienas buvo 0,10% ($P < 0,05$) riebesnis, lyginant su AA genotipo karvėmis. Beta laktoglobulino lokuso BB genotipo karvių pienas 0,20% ($P < 0,001$) buvo baltymingesnis, nei AA genotipo karvių pienas.

Įdiegus pieno baltymų beta laktoglobulino ir kapa kazeino genų atžvilgiu tyrimo metodiką galima genetiškai sertifikuoti veislinius gyvulius – rinktines karves, bulius bei jų spermą pagal genus, lemiančius pieno baltymų kokybinę sudėtį, šitaip padidinti jų veislinę vertę bei sudaryti galimybes genetiškai pagerinti pieno kokybę.

Raktažodžiai: kazeinas, laktoglobulinas, pienas, galvijai

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ИССЛЕДОВАНИЕ ГЕНЕТИЧЕСКИХ ФАКТОРОВ, ВЛИЯЮЩИХ НА СОСТАВ МОЛОЧНОЙ ПРОДУКЦИИ КОРОВ ЛИТОВСКОЙ ПОРОДЫ

Резюме

Пробы крови получены у 394 неродственных коров литовской молочной породы для исследования генов молочного белка, контролирующих молочную продуктивность. Из них: 109 литовской черно-пестрой, 168 литовской бурой, 68 литовской пепельной, 49 литовской белоспинной. Для исследования генов генотип молочного белка идентифицирован методом ПЦР (полимеразная цепная реакция). Установлено, что генотип ВВ каппа-казеин положительно влиял на количество белков и жиров в молоке и отрицательно – на выход молока. Генотип ВВ альфа$_{S1}$-казеин влиял на увеличение выхода молока, при этом генотип СС влиял на увеличение содержания белка в молоке. Белок сыворотки генотипа ВВ бета-лактоглобулин значительно влиял на жиры, при этом генотип ВС влиял на увеличение белка в молоке. Анализ данных показал, что коровы с генотипом АА в среднем дали на 175,7 кг ($P < 0,05$) больше молока и на 2,88 кг ($P < 0,001$) больше белков, чем коровы с генотипом АВ. При этом, в молоке коров с генотипом АВ жиров было на 0,23% ($P < 0,001$) и белков на 0,06% ($P < 0,001$) больше. В молоке коров, имеющих локус каппа-казеина генотипа ВВ, в среднем жиров на 0,27% ($P < 0,05$) и белков на 0,21% ($P < 0,001$) больше, чем в молоке коров с генотипом АА. При сравнении генотипов ВВ и СС локуса альфа$_{S1}$-казеина выявлено, что коровы генотипа ВВ дали молока на 1344 кг ($P < 0,05$) больше, но в молоке коров с генотипом СС белков было больше на 0,35%. При сравнении генотипов локуса бета-лактоглобулин установлено, что коровы генотипа АА уступают другим: коровы генотипа АВ в среднем дали молока на 332,2 кг ($P < 0,01$) больше (и более жирного), а в молоке коров генотипа ВВ белков было на 0,20% больше ($P < 0,001$) молоко белковомолочнее, чем молоко коров АА генотипа. На основе созданной методики исследования молочного белка бета-лактоглобулин и каппа-казеин можно генетически сертифицировать породистых животных – отобранных коров и быков, а также их сперму – по генам, положительно влияющим на качественный состав молочного белка, и таким способом повысить породистую ценность скота, создавая возможность генетически улучшить качество молока.

Ключевые слова: казеин, лактоглобулин, молоко, скот