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Influence of κ-Casein Genotype on Milk Productivity of Latvia Local Dairy Breeds

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Abstract**—**κ-casein is one of milk proteins which are very important for milk processing. Genotypes of κ-casein affect milk yield, fat, and protein content. The main factors which affect local Latvian dairy breed milk yield and composition are analyzed in research. Data were collected from 88 Latvian brown and 82 Latvian blue cows in 2015. AA genotype was 0.557 in Latvian brown and 0.232 in Latvian blue breed. BB genotype was 0.034 in Latvian brown and 0.207 in Latvian blue breed. Highest milk yield was observed in Latvian brown (5131.2 ± 172.01 kg), significantly high fat content and fat yield also was in Latvian brown ($p < 0.05$). Significant differences between κ-casein genotypes were not found in Latvian brown, but highest milk yield (5057 ± 130.23 kg), protein content (3.42 ± 0.03%), and protein yield (171.9 ± 4.34 kg) were with AB genotype. Significantly high fat content was observed in Latvian blue breed with BB genotype (4.29 ± 0.17%) compared with AA genotypes (3.42 ± 0.19). Similar tendency was found in protein content – 3.27 ± 0.16% with BB genotype and 2.59 ± 0.16% with AA genotype ($p < 0.05$). Milk yield increases by increasing parity. We did not obtain major tendency of changes of milk fat and protein content according parity.

Keywords**—**κ-casein, polymorphism, dairy cows, milk productivity.

I. Introduction

THE milk production traits in cattle are quantitative traits being influenced by environmental and genetic factors. Milk yield and it composition is the primary goal for animal selection in dairy industry. Casein is a family of milk proteins, that exists in different molecular forms [3], [14]. Kappa-casein gene is situated on bovine chromosome 6, and its polymorphism has been known since 1964. 11 alleles have been found, but A, B, and E are the most popular [15], [30]. The A and B variants are different in the two amino acids positions. Threonine is 136 position and aspartic acid is 148 position in A variant, while isoleucine and alanine are in B variant, respectively. [2], [14]. Allele A has been found as the most frequent, with the effect of increasing milk yield, but decreasing protein content. Allele B increases protein content and milk coagulation properties. Negative effect on milk coagulation properties was observed by allele E [4], [17]. Tsiaras et al. [37] observed only AA and AB genotypes of Holstein with frequencies of 0.89 and 0.11, respectively, but allelic frequencies were 0.94 and 0.06 for A and B, respectively. Cows with genotype AB produced milk with significantly higher protein yield and content. A and B allelic frequencies of Jersey breed were 0.32 and 0.68, but frequencies of AA, AB, and BB genotypes were 0.21, 0.23, and 0.56, respectively [31].

Latvian brown (LB) breed began to be formed in the middle of the last century when Angler cattle were imported into Latvia to improve the local, low-productive cattle. The crosses had a higher milk yield but lower fat content. At the end of the last and early in this century, Danish Red bulls were imported and used on the Angler crosses and on the local cattle [13]. According to milk record, LB (all LB cows with different bloodiness) milk yield was 6329 kg with 4.35% fat and 3.34% protein content in 2015 [6].

Latvian blue (LZ) breed also is a local breed. The color is blue - gray. Same animals can be light grey or dark blue. The legs have a darker color than body, and blue ring can be around the eyes. Same cows have a lighter head, face and temporal part of nasal mirror is dark gray or black. Cows are suitable for grazing in pastures [34]. Milk yield of LZ was 5134 kg with 4.25% fat and 3.34% protein content in 2015. The highest milk yield is key aspect about why farmers choose Holstein breed, ignoring lower fat and protein content (9066 kg with 3.87% fat and 3.34% protein content). Number of LB and LZ decreases although these breeds are preserved as animal genetic resources. It is very important to find strong positive arguments for these breeds. Suitable milk for cheese making and processing could be important factor with effect LB and LZ preservation [6].

Milk is an important source of high-quality proteins and fats, and dietary energy. Milk components provide a part of human daily nutrition. [22]. The milk crude protein contains specific protein fractions. The main group of milk proteins is the casein (κ, β, α_{s1}, α_{s2}), it constitutes about 78 to 82% of bovine milk proteins [7]. Caseins contain suitable amino acid composition for growth and development of the newborn [24]. This high-quality protein in cow milk is one of the key reasons why milk is so widespread in food industry. Increasing casein content increases cheese yield and ability of milk coagulation [9], [21]. Kappa-casein plays an important role in the formation, stabilization, and aggregation of the casein micelles, thus altering the manufacturing properties and digestibility of milk [19], [38].

The purpose of research was to analyze the influence of κ-casein genotype, breed, and parity on milk yield and composition in local Latvian dairy breeds.

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II. Materials and Methods

A. Data Collection

Data were collected from 88 LB and 82 LZ (*Bos taurus*) cows in 2015. All these cows are animal genetic resources (bloodiness of LB and LZ >50%). Cows were selected randomly. Cows were grazing in the summer, but feeding with hay, haylage, and grains in winter. Cow's dislocation is extended to the whole Latvia.

Grouping according to the parity number of 1st parity cows was 170, number of 2nd parity was 129, and number of 3rd parity was 87. Grouping according to κ-casein genotype, number of AA genotype was 154, number of AB genotype was 180, and number of BB genotype was 47, number of AE genotype was 4, and number of BE genotype was 1. Evaluating the impact on milk yield and composition, we did not use genotypes AE and BE due to the small numbers of animals.

B. Blood Sample Collection and Analysis

κ-casein genotypes were identified using Polymerase Chain Reaction and Restriction Fragment Length Polymorphism (PCR-RFLP) and electrophoresis on 3% agarose gel. The identification of CSN3 single nucleotide polymorphisms (SNPs) was done according to Velmala et al. [39]. SNPs at positions 13104 and 13124 were examined to determine the nucleotide changes (A->C and A->G), which determine CSN3 alleles A, B, and also E. For digestions, endonuclease *HinfI* was used to detect the presence of alleles A and B, and endonuclease *BsuRI* to detect the presence of allele E.

The research of CSN3 polymorphisms started from 2015. Identification of CSN3 genotypes was done in Scientific Laboratory of Molecular Biology and Microbiology of Latvia University of Agriculture.

C. Milk Analysis

Information about milk yield and composition in 305 days were collected from Latvian Agriculture Data processing center, but milk samples were analyzed in an accredited milk quality laboratory Ltd. "Piensaimnieku Laboratorija" according ISO standards: milk fat – ISO 9622/IDF 141, milk protein – ISO 9622/IDF 141, somatic cell count – LVS EN ISO 13366-2.

D. Statistical Analysis

For data processing, SPSS program was used. The effect of κ-casein genotype, parity and breed on milk yield 305-days, fat and protein content, fat and protein yield and somatic cell score were analyzed using the following model: Y_{ijk} = mean + $G_i + P_j + B_k + e_{ijk}$ where Y_{ijk} = milk yield, fat content, protein content, fat yield, protein yield, somatic cell score, G_i = genotype of κ-casein (i=1,2,3), P_j = parity (j=1,2,3), B_k = breed (k=1,2), e = random residual effect.

Least square means and standard error (LSM±SE) were input in the tables. Somatic cell count (SCC) was calculated to somatic cell score (SCS) [33].

Differences between groups of breeds, κ-casein genotypes, and parities were used for multivariate variance with Bonfferoni Post Hoc test.

Significant differences (p<0.05) in the tables were marked with different superscripted letters of alphabet (a, b).

III. Results and Discussion

Allelic frequencies of A, B, and E were 0.761, 0.227 and 0.011 in LB, and 0.506, 0.488 and 0.006 in LZ, respectively. Frequencies of AA, AB, BB, AE genotypes were 0.557, 0.386, 0.034, and 0.023 in LB. AB genotype of LZ was higher compared to LB – 0.549, respectively. We did not find AE genotype in LZ (Table I).

An allele and AA genotype is widespread in Holstein, South Anatolian red, East Anatolian red, Turkish grey. Allelic frequencies of A were 0.628 to 0.841 in the above breed, but AA genotypes varied from 0.340 to 0.727. Highest allelic frequency of B was observed in Brown Swiss, 0.557 [1]. B allele in the Holstein breed was found in the range between 0.06 and 0.57 [10], [20]. Allele E was rare in other papers also, for example, allelic frequency of E allele was 0.03 in Simental cows [8]. Allelic frequency of A allele was 0.81 in Iranian native breed, while in Iranian Holstein it was 0.59 and it provides that not all native dairy breeds are with high potential for cheese making [14].

TABLE I
Frequencies of Alleles and Genotypes

Alleles and genotypes	Breed	
	LB (n = 88)	LZ (n = 82)
A	0.761	0.506
B	0.227	0.488
E	0.011	0.006
AA	0.557	0.232
AB	0.386	0.549
BB	0.034	0.207
BE	0.000	0.012
AE	0.023	0.000

Highest milk yield was observed in LB – 5131.2±172.01 kg (Table II). Milk yield of LZ was 4967.7±131.81 kg. Highest fat and protein content was in LB – 4.43±0.15% and 3.30±0.12%, respectively. Fat content and fat yield was significantly different between LB and LZ (p<0.05). Milk yield of local dairy breed usually is less compared with modern and intensive dairy breed. Explanation of that tendency could be associated with housing condition and feeding system, not only with genetic potential. Local breeds in most cases were kept in small farms with extensive farming. Previous studies proved that milk yield of Latvia local breed is significantly lower compared to intensive dairy breed, e.g. Holstein. Fat and protein content could be highest depending on the animal health, feeding, stage of lactation, lactation number [28].

Lithuanian Light Grey and Lithuanian White Backed were local dairy breeds in Lithuania. Milk yield of these breed was lower compared to the modern dairy breeds – Lithuanian Black and White and Lithuanian Red. Average 305-day daily milk yield of Light Grey and Lithuanian White Backed was

15.4 kg and 14.8 kg, respectively [5], [36].

TABLE II
MILK YIELD AND COMPOSITION ACCORDING BREED

Traits	Breed	
	LB	LZ
Milk yield, kg	5131.2±172.01	4967.7±131.81
Fat content, %	4.43±0.15^{a}	3.97±0.12^{b}
Fat yield, kg	227.9±10.80^{a}	189.9±8.28^{b}
Protein content, %	3.30±0.12	3.03±0.09
Protein yield, kg	168.4±8.18	149.3±6.24
SCS	2.81±0.27	2.41±0.21

^{a,b} – traits with different letters in superscript are significantly different between breeds ($p<0.05$)

Highest milk yield that we observed in group with AB genotype was 5057.9±130.23 kg, but lower milk yield was in BB group, 4642.3±416.95 kg in LB. Highest fat content was in AB genotype group (4.59±0.05%), but lower in BB genotype group. We did not find significant effect on milk yield and composition in LB (Table III). Similar tendency was find in previous papers about κ-casein genotype effect on LB and LZ milk yield and composition [35]. Milk protein content and highest yield of Holsteins was observed in AB κ-casein group. Compared to AA genotype, these traits were +0.08±0.04% and +21±9 kg, respectively [37]. Increased protein yield and content associated with the AB κ-casein genotype have been found in the other studies [26], [27]. Contradictory results were investigated by Chrenek et al. [11]; highest milk yield, fat, and protein content were found in BB genotype in Brown Swiss.

TABLE III
MILK YIELD AND COMPOSITION ACCORDING GENOTYPE IN LB

Traits	Genotype		
	AA	AB	BB
Milk yield, kg	4860.6±111.44	5057.9±130.23	4642.3±416.95
Fat content, %	4.57±0.05	4.59±0.05	4.17±0.17
Fat yield, kg	223.1±5.14	231.4±6.00	193.3±19.22
Protein content, %	3.38±0.02	3.42±0.03	3.36±0.08
Protein yield, kg	164.2±3.71	171.9±4.34	155.4±13.89
SCS	2.01± 0.17	2.53±0.20	2.90±0.63

Highest milk yield was in AA genotype (5041.9 ± 160.49 kg) in LZ. Significantly high fat and protein content was observed in BB genotype group ($p<0.05$). Similar tendency was in fat and protein yield. Highest SCS was in BB genotype (Table IV).

Previous researchers obtained that AB genotype might be associated with higher milk yield rather than the AA genotype; in our investigation, we did not find that tendency [34], [7]. Milk yield in Bulgarian Rhodopean was 4099 kg with AB genotypes and it was highest compared to AA and BB genotypes [16]. Other results accepted that milk production of BB homozygous cows was 15% lower compared with AB heterozygous cows. Latvian researchers Paura and Jonkus observed average milk yield 4158 ± 157.8 kg and 3995 ± 162.4 kg with AA and AB genotypes in LB breed. Protein content was the same with both genotypes – 3.33%. Average fat content was 4.51±0.04% and 4.51±0.02% [27].

TABLE IV
MILK YIELD AND COMPOSITION ACCORDING GENOTYPE IN LATVIAN BLU

Traits	Genotype		
	AA	AB	BB
Milk yield, kg	5041.9±160.49	4704.4±105.07	4979.3±166.55
Fat content, %	3.42±0.19^{a}	3.95±0.12	4.29±0.17^{b}
Fat yield, kg	164.4±12.46	182.5±8.16	201.5±12.93
Protein content, %	2.59±0.16^{a}	3.11±0.10^{b}	3.27±0.16^{b}
Protein yield, kg	137.8±9.69	143.9±6.34	154.1±10.05
SCS	2.58±0.26	2.55±0.17	3.35±0.27

^{a,b} – traits with different letters in superscript are significantly different between genotypes ($p<0.05$)

Parity effected milk yield and composition. Highest milk yield was observed in 3rd parity, respectively, 5158.0 to 5803.6 kg in LB and 4922.9 to 6150.1 kg in LZ, but lower in 1st parity. Highest fat content was in LB in all genotypes and parities. Peak value of fat content of LB was in 2nd parity in AA genotypes (4.69%). Highest protein content also was in LB compared with LZ. We did not find confirmation that BB genotype determinates highest protein content, protein content of LZ was significantly lower in 3rd parity in AA genotype (2.27%; $p<0.05$), (Fig. 1).

Parity effect on milk yield and composition was found in previous studies [18], [25], [29]. Milk yield per parity, milk yield per day, milk yield per calving interval, efficiency of milk production and cow weight at calving increased with increased parity. Cows in 1st parity yielded less milk per lactation, than 2nd, 3rd, and 4th parity ($p<0.05$) [32]. Different result was found by M'hamdi et al. [23]. Milk yield increasing until 2nd parity and in next parities milk yield decreased. Same researchers observed tendency that milk yield was lower ($p<0.05$) in 1st parity than increasing until the 5th parity, and then, it decreases [41].

Different researchers obtained different results about parity influence on milk composition. Węglarz et al. [40] did not find significant effect on milk composition by parity, but Czaplicka et al. [12] reported the increase of milk yield and the propo rtional increase of fat, protein, lactose long with the increase of cows' age.

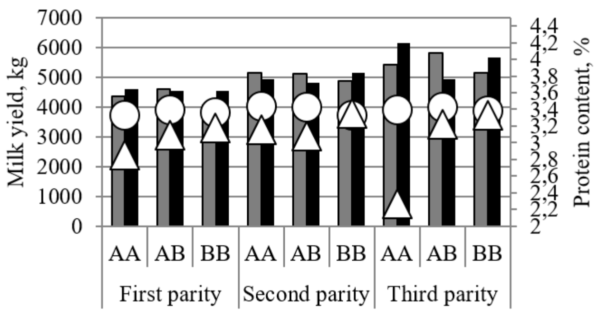

Fig. 1 Genotype, parity and breed influence on milk yield and protein content: ■ – milk yield of LB, kg, ■ – milk yield of LZ, kg, ○ – protein content of LB, %, Δ – protein content of LZ,%

Genomic selection started at 2009, and nowadays this technology is developing and distributing rapidly. The development of genomic selection increases dairy cows genetic potential growing. It allows achieving high accuracy and reducing time of animal evaluation. Genes of milk protein are important for the dairy cow selection. Researchers need to find the strengths in local breed, e.g. to breed sires with BB κ-casein genotype.

Daughters of one sire may perform differently in large herds compared small herds, or in herds with a total mixed ration compared herds with a hay feeding system. Environmental factors effected the cows' health, welfare, and milk yield and composition. It is important to ensure the best housing conditions and then cows can to represent their genetic potential [42].

IV. Conclusion

Kappa-casein genotypes affect milk yield and composition. Milk yield is very important, but milk fat and protein content and yield should not be forgotten. Milk protein composition and content affect ability of milk processing. AA genotype is widespread in LB and LZ breed. Frequencies of BB genotype were 0.034 and 0.207, respectively. Milk yield was highest in LB. Highest fat and protein content also was in LB breed. According to genotype, highest milk yield was with AB (LB) and AA genotype (LZ). Highest fat content and protein content was with BB genotype in LZ. Increasing parity, milk yield increases.

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