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The influence of k-casein genotype on the coagulation properties of milk collected from the local Latvian cow breeds

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Abstract. Cheese production is becoming increasingly more diversified all over the world. The information on milk coagulation properties among Latvian dairy cow breeds and its suitability for cheese production still remains unclear. At the same time, milk with good renneting properties collected from the native Latvian cows can be used for the production of Protected Denomination of Origin (PDO) cheeses. The purpose of this research was to analyse the influence of the milk protein genotypes present in Latvian native cattle breeds on the milk coagulation properties. The Data was collected in 2016 from 56 Latvian brown, 26 Latvian blue and 13 Holstein black and white cows i. Highest frequency of AA κ-casein genotype was found in Latvian brown breed (0.593), while AB genotype was more often present in the Latvian blue breed (0.636). It has been found that the presence of κ-casein genotype resulted in no significant difference in milk composition and milk coagulation properties among studied cattle breeds. We have observed a tendency that the most desirable milk coagulation properties were present in BB genotype. A significant effect of breed on milk composition has been found ($p < 0.05$). Milk yield of Holstein Black and White was 32.0 ± 2.99 kg, while in Latvian blue it was only 17.6 ± 1.32 kg. Higher milk yield was obtained in Latvian blue breed in comparison to that of the brown breed – 19.10 ± 0.76 kg. Better milk coagulation properties were observed in Latvian brown breed – shorter milk renneting time (16.86 ± 1.15 min), highest curd yield (24.0 ± 0.79%) and curd firmness (3.21 ± 0.17 N).

Key words: Latvian brown cows, Latvian blue cows, milk coagulation properties

INTRODUCTION

The production of cheese has grown in recent years and cheese plays an important role in the economics of Latvian dairy industry. The technological properties of individual milk samples for cheese making have traditionally been analysed through the assessment of milk coagulation properties.

Milk coagulation properties are influenced by various factors, whilst the animal breed remains the most important factor (Malchiodi et al., 2014). The B allele of κ-casein is associated with more desirable coagulation properties and higher protein content in milk (Azevedo et al., 2008). A allele increases milk yield and decreases protein content in it. A and B alleles are prevalent compared to E allele. Negative effect of E allele on milk coagulation properties (more non-coagulated milk samples, longer milk renneting time and lower curd yield) were found in previous studies (Davoli et al., 1990; Macheboeuf et al., 1993; Ikonen et al., 1999).

The frequencies of genotypes and alleles of κ-casein vary across the breeds AA genotype and A allele are occurring in high-productivity dairy breeds (Tsiaras et al., 2005; Bonvillani et al., 2010). Choobini et al., (2014) have found that AA genotype frequency was 0.174 in Holstein, while AB and BB genotypes frequencies were 0.304 and 0.261, respectively. Genotypes with E allele were with low frequencies, AE and EE genotypes were found with frequencies 0.043 and 0.087 (Choobini et al., 2014). Frequencies of A allele were 0.790 and 0.695 in Estonian Holstein and Estonian Native, while frequencies of B allele were 0.138 and 0.305, respectively (Jõudu et al., 2007).

A preliminary comparison of milk composition among Latvian dairy breeds in earlier studies (Cielava et al., 2015) shows that the milk collected from the Latvian native cows has a number of advantages, despite the fact that the limited numbers of studies are available on the suitability of Latvian native cow's milk. The purpose of this research was to analyse the influence of κ-casein polymorphism present in Latvian native cattle and Holstein Black and White breeds on the milk coagulation properties.

MATERIALS AND METHODS

Individual milk samples (n = 95, morning milking) from the Latvian cow breeds (56 Latvian brown (LB), 26 Latvian blue (LZ) and 13 Holstein black and white cows (HBW) were collected across different Latvian regions during the summer of 2016 July to August, 2016) period.

Cows were kept in small and extensive farms – on average 7 animals in each farm. During the summer cows were grazed in cultivated pastures, fed with grain meals and minerals. LB and LZ cows according bloodiness were animal genetic resources in Latvia. LB cows were with ≥ 50% LB bloodiness and the rest were Danish red, Angeln bloodiness. LZ cows were with ≥ 50% LZ bloodiness and rest was Lithuanian grey, Tyrol, Latvian brown. HBW cows were with Holstein bloodiness 75 to 90% and rest was Latvian brown, Holstein Red and White bloodiness. Cows with AA genotype were 134 ± 12.6 days in milk, with AB genotype 130 ± 13.5 days in milk, but cows with BB genotype 139 ± 14.1 days in milk. Milking was done two times per day automatically. Cows with AA genotype were 2.9 ± 0.25 parity, cows with AB genotype 2.7 ± 0.34 parity, while cows with BB genotype 3.1 ± 0.56 parity. Live weight of LB, LZ and HBW was 557 ± 10.6 kg, 520 ± 12.4 kg and 620 ± 6.5 kg, respectively.

Approximately 120 LB and 100 LZ cows were included in animal genetic resources savings programme in Latvia. Milk samples were taken from a number of those cows and analysed to determine the coagulation properties of milk produced by the native Latvian cows.

Blood samples for *CSN3* genotyping were analysed in Scientific Laboratory of Molecular Biology and Microbiology of the Latvian University of Agriculture (LUA). Blood samples were collected from jugular vein (*Vena Jugularis*) in 10 mL sterile vacuumtainers. Genotyping was done 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. (1993). 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 were used endonuclease *HinfI* to detect the presence of alleles A and B, and endonuclease *BsuRI* to detect the presence of the allele E.

Protein and fat content, curd yield and curd firmness were detected in Faculty of Food Technology of LUA. Protein content was detected according to ISO 8968-1:2014 using Kjeltec^{TM} 2100 (Foss, Denmark). Fat content was detected according to ISO 488:2008 using centrifuge (Funke Gerber, Germany).

Curd yield (%) was calculated by weight of curd obtained from milk.

The rennet (CHY-MAX 1000 IMCU/ml, Chr. Hansen, Denmark) used in the analyses of curd firmness was diluted 1:100 (v/v) and added 0.2 ml to 10 ml of milk. The curd firmness (in Newtons) was determined after 30 min of milk renneting at 35 °C using Texture Analyser TA-HD plus (Stable Micro System, UK). Compression method for determination of curd firmness (technical data – disc A/BE – d45, test speed 1.0 mm s^{-1}, distance in the depth of curd sample 8 mm) was used.

Renneting time in minutes was analysed using 1:100 (v/v) of rennet solution into water and measuring the time until flocculation of milk was started at 35 °C. For interpretation of results, all samples were divided into four groups (fast, average, slow and non-coagulating milks) based on the time devoted for the clotting of samples. The assessment of clotting time was as follows: fast = flocculation formation during 10 min, average = 15 min, slow = more than 15 min, and non-coagulating = samples that did not coagulate at all.

Statistical data processing was carried out using MS Excel and SPSS 15.0 for Windows. The differences between group were significant if $p < 0.05$.

RESULTS AND DISCUSSION

Allelic frequencies of A, B, E alleles were 0.778, 0.213 and 0.009 in LB breed. The frequency of B allele was higher in LZ breed – 0.364, respectively. Allele E was not found in LZ and HBW breed. A low frequency of E allele presence was observed in Latvian cow breeds. Researchers from different countries also confirms low frequencies of E allele (Lien et al., 1999; Zepeda-Batista et al., 2014)

The frequency of A allele increases in high-productivity animal selection. Genome selection is the instrument of increasing the frequency of B allele – focusing on dairy breeds that produce milk suitable for cheese production (Kübarsepp et al., 2005). Frequency of B allele in Lithuanian dairy cows was 0.23 higher comparing to that of Latvian breeds (Morkūnienė et al., 2016). Smiltina and Grislis (2010) have observed the frequency of AA genotype equal to 0.467 in LB and 0.465 in LZ breed, while frequencies of AB genotype were 0.433 in LB and 0.0437 in LZ.

Genotype frequency of AA was higher in LB breed compared with LZ breed – 0.593 and 0.318, respectively. Frequency of genotype BB was highest in LZ breed. Frequency of AA genotype was 0.615 in HBW, while frequency of AB was 0.385 (Table 1). According to previous research (Tsiaras et al., 2005), AA genotype is widespread in Holstein breed. Frequency of AA genotype was observed 0.89, while frequency of AB was only 0.11 in Holstein.

We did not analyse the influence of AE κ-casein genotype on milk productivity owing to low number of cows with this genotype in our sample.

Milk coagulation properties determine the quality of cheese produced from that milk yield. We have found a significant influence ($p < 0.05$) of κ-casein genotype on milk productivity, milk quality and milk coagulation properties. We have found higer milk yield in AA genotype, while lower milk yield was in BB genotype group – 19.3 ± 0.86 and 17.1 ± 3.61 kg, respectively. Notably, Lithuanian researchers have similarly found that κ-casein genotype significantly influences the milk yield from dairy cows. Highest milk yield from cows with AA genotype, compared with AB and BB κ-casein genotypes, was found in Lithuanian dairy cows (Pečiulaitienė et al., 2007).

Table 1. κ-casein alleles and genotypes frequencies in analysed dairy cows

Alleles and genotypes	Frequency of alleles and genotypes		
	Latvian brown (*n* = 56)	Latvian blue (*n* = 26)	Holstein Black and White (*n* = 13)
A	0.778	0.636	0.808
B	0.213	0.364	0.192
E	0.009	–	–
AA	0.593	0.318	0.615
AB	0.352	0.636	0.385
BB	0.037	0.045	–
AE	0.019	–	–

Similar results were obtained in Sitkowska and co-authors (2013) study on κ-casein genotype influences on milk protein content. Highest protein content was observed in BB and AB genotype group – 3.47 ± 0.24% and 3.46 ± 0.83%, respectively.

Shorter milk renneting time was observed in BB genotype group 13.31 ± 1.84 min, whilst the longest time was observed in AA genotype group ($p < 0.05$, Table 2)

In the current research all milk samples were distributed in the following manner: fast flocculation was 15%, average – 30%, slow – 47%.

We observed 8% non-coagulated milk samples, mostly from cows with AA genotype (two samples were from AB genotype and six from AA genotype). Three non-coagulated samples were from HBW and LZ breed, two from LB. Similar results were found in previous researches, Ikonen et al. (2004) and Cassandro et al. (2008) reported that 7.5% to 13.2% Holstein cow's milk samples are not coagulated. Kübarsepp et al. (2006) has found that 4.42% non-coagulated milk came from cows with AE κ-casein genotype and 4.04% from AA genotype cows. Milk from cows with BE and BB genotype was not coagulated (only 1.22% and 1.36%). Zannoni and Annibaldi (1981) recommended that optimal value of milk coagulation time is 13 min and whilst we have obtained similar results only in the BB genotype group – 13.31 ± 1.84 min.

Curd yield is a very important parameter for cheese production. It influences the quality and costs of product. Highest curd yield was in BB genotype group – 28.5 ± 3.26%, while the lowest was observed in AA genotype group ($p < 0.05$). Ng-Kwai-Hang and co-authors (1984) have confirmed that milk obtained from cows with BB genotype had the highest curd yield compared to the milk collected from cows with AA and AB κ-casein genotype Curd yield can be explained by the difference in cows' genotype i.e. cows with AA genotype have a higher proportion of large casein micelles (average 206 nm), which reduces the curd yield efficiency and it is the explanation of lower curd yield. Burbano and collages (2010) have also found that the highest curd yield was from cows with BB κ-casein genotype, the average curd yield was observed in AB genotype, while the lowest curd yield was obtained from cows with AA genotype.

Curd formation time and its firmness plays an important role in the cheese production process. Significant differences were not found in curd firmness, however by analysing individual samples we have found that the highest curd firmness was in AA and AB genotype group (3.22 N in both group).

Table 2. Milk productivity and milk coagulation properties by κ-casein genotype

Trait	AA (n = 52)	AB (n = 40)	BB (n = 3)
Milk yield, kg	19.30 ± 0.86	18.40 ± 1.25	17.10 ± 3.61
Fat content, %	4.26 ± 0.40^{a}	3.61 ± 0.22^{b}	4.07 ± 0.61
Protein content, %	3.39 ± 0.15	3.46 ± 0.83	3.47 ± 0.24
Milk renneting time, min*	18.47 ± 1.78^{a}	16.54 ± 2.06^{ab}	13.31 ± 1.84^{b}
Curd yield, %	21.10 ± 2.13^{a}	23.30 ± 1.15^{ab}	28.50 ± 3.26^{b}
Curd firmness, N	3.22 ± 0.50	3.22 ± 0.25	3.07 ± 0.72

^{a,b} – traits with different letters in superscript are significantly different between breed ($p < 0.05$)
* data includes only coagulated samples (n = 87)

The breed of dairy cows has considerably influenced the quality and yield of milk ASignificantly higher milk yield was observed in HBW group - 32.0 ± 2.99 kg, compared with LB and LZ groups (Table 3; $p < 0.05$).

Highest fat content was observed in LB group – 4.47 ± 0.13%, while lowest in HBW group 2.21 ± 0.20%, respectively. Fat content was significantly different between all groups ($p < 0.05$). Petrovska and Jonkus (2014) revealed that milk fat and protein content between LB genetic resources and HBW was not significantly different, whilst milk yield was significantly highest in HBW. However, Paura et al., (2012) have observed significantly higher milk fat content from LB compared with HBW breed in middle lactation stage ($p < 0.05$).

Table 3. Milk productivity and milk coagulation properties by breed

Trait	Latvian Brown (n = 56)	Latvian Blue (n = 26)	Holstein Black and White (n = 13)
Milk yield, kg	19.10 ± 0.76^{a}	17.60 ± 1.32^{a}	32.00 ± 2.99^{b}
Fat content, %	4.47 ± 0.13^{a}	3.53 ± 0.23^{b}	2.91 ± 0.20^{c}
Protein content, %	3.45 ± 0.05	3.50 ± 0.09	3.43 ± 0.07
Milk renneting time, min*	16.86 ± 1.15^{a}	18.33 ± 3.19^{a}	27.17 ± 4.56^{b}
Curd yield,%	24.00 ± 0.79^{a}	22.50 ± 0.98^{ab}	18.70 ± 1.09^{b}
Curd firmness, N	3.21 ± 0.17	3.34 ± 0.22	2.64 ± 0.43

^{a,b,c} – traits with different letters in superscript are significantly different between breed ($p < 0.05$)
* data about samples witch were coagulated (n = 87)

Protein content of mil did not vary significantly among the analysed cow breeds Highest protein content was in LZ group – 3.50 ± 0.09%, while lower protein content in HBW breed. According to De March and co-authors breed affects milk fat and protein content significantly. Holstein is a high-yield breed that produces milk with significantly lower fat and protein content compared with local dual-purpose breeds such as Alpine Brown, Simmental, Alpine Gray (De Marchi et al., 2007).

Significantly different milk renneting time was observed in HBW breed – 27.17 ± 4.56 min. Favourable conditions for milk renneting positively affect curd formation, curd firmness, whey syneresis and cheese ripening processes (Cassandro et al., 2008). Shorter renneting time was observed in LB (16.86 ± 1.15) and LZ (18.33 ± 3.19) breed cow's milk ($p < 0.05$). Varotto et al. (2014) have found that native dairy cow breeds produce milk with shorter milk renneting time comparing to HBW. Significantly higher curd yield (24.0 ± 0.79%) was observed in LB breed. Significant difference ($P < 0.05$) was established between HBW and LB breeds

Positive phenotypic correlation coefficient was observed between protein content and curd firmness and it has varied from 0.539 to 0.609. Similar tendency was found in Kübarsepp et al. (2005), where correlation coeffient of 0.310 was obtained. Negative correlation coeficient was present between protein content and milk renneting time (varieted from -0.490 to -0.510). Weak correlation was observed between milk renneting time and curd firmness (Fig. 1). Cassandro et al. (2008) found a strong phenotypic correlation (-0.76) between milk renneting time and curd firmness i.e. when milk renneting time decreases, curd firmness increses.

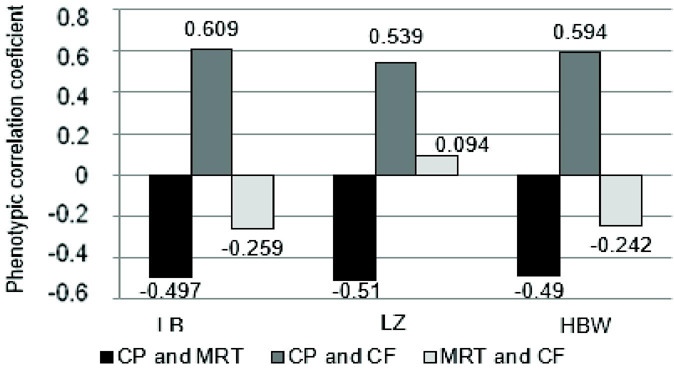

Figure 1. Phenotypic correlation coefiecients between CP and MRT (protein content, % and milk renneting time, min), CP and CF (protein content,% and curd firmness, N), MRT and CF (milk renneting time, min and curd firmness, N).

According to the previous research from Ostersen and co-authors (1997), milk coagulation properties are affected by different factors such as milk quality, breed, season, and herd. Milk chemical composition and coagulation properties were also significantly affected by stage of lactation (Zendri et al., 2017). Therefore for better understanding and explanation of breed and genotypes potential for cheese making further studies on milk protein profiles and genetic variants of different protein fractions during different seasons should be done.

CONCLUSIONS

AA κ-casein genotype was more widespread in LB breed, while AB genotype was with highest frequency observed in LZ breed. Highest values of milk yield and milk fat content were observed in AA genotype group. Highest protein content and better coagulation properties were found in BB genotype group.

Significantly lower milk yield was obtained from LB and LZ breed, while milk fat content was significantly lower in HBW breed.

Most favourable milk coagulation properties were detected in BB genotype – highest curd yield, curd firmness and shorter milk renneting time.

In order to define an appropriate breed and κ-casein genotype for cheese making, further researches that takes into consideration other factors which affect milk composition and coagulation properties needs to be done

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