

Study on milk composition and milk protein distribution in Romanian Holstein cattle

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ABSTRACT

The aim of this study was to determine milk quality indices as well as the milk protein composition in Romanian Holstein cattle raised under the conditions of experimental farm of INCDBNA-IBNA. The study was carried out on 22 milk samples. The types of different milk proteins were identified by SDS-PAGE technique. Sampling day and milk chemical composition were performed during the milking period of studied cattle. The quality indices were breed-specific for protein (3.38%) and higher for fat (4.39%).

Milk proteins analysis of Romanian Holstein cattle separated by SDS-PAGE electrophoresis showed the presence of four major caseins (α 1-, α 2-, β - and k-casein) and two whey proteins (β -lactoglobulin, α -lactalbumin). The caseins accounted 77.28% of the total milk proteins, while the major proteins (β -lactoglobulin, α -lactalbumin) from the whey represented 22.72% of the total proteins. α 1-casein + α 2-casein had a higher expression (36.01%) followed by β -casein (31.45%), β -lactoglobulin (18.16%), k-casein (9.82%) and α -lactalbumin (4.56%). The most of milk samples was characterized by a medium expression level of both caseins and whey proteins

Keywords: cattle, composition, milk quality indices

INTRODUCTION

The Romanian Holstein breed is a milk-oriented cattle breed. Milk production traits (milk volume, fat and protein yields, fat content and protein content) are important quantitative traits, which had tremendous influence of dairy industry (Zhang et al., 2007).

For example, the proportions of proteins in milk play an important role in determining the functional properties of milk, such as clothing and cheese yield (Wedholm et al., 2006).

Previous analysis has shown variation in milk composition (Schopen et al., 2009).

Yang et al., (2013) have shown that the breeds had evident effect on milk cattle composition and physicochemical properties.

Maurmayr et al., (2018) have shown that compared with purebred Holsteins, cows crossbred from Swedish Red, Montbeliarde and Brown Swiss sires yielded milk with a protein composition more favorable to cheese production. The modification of the proportion, content and daily yield of milk protein fractions may have very important nutritional, technological and economic implications (Maurmayr et al., 2018).

For the analysis of the milk proteins composition different authors were used the method SDS-PAGE electrophoresis.

Milk protein fractions were influenced by herd-test-day, stage of lactation, parity and breed combination (Maurmayr et al., 2018).

Herd-test day significantly affected all milk protein fraction contents and proportions in particular α s1-casein and α -lactalbumin, which might reflect the effect of difference in the environmental, management and feeding conditions across herds and variations in the test records collected on different days (Maurmayr et al., 2018).

The aim of this study was to determine the milk quality parameters and to study milk protein composition in a cow population belonging to Romanian Holstein breed, a milk-oriented cattle breed for further genetic improvement.

MATERIALS AND METHODS

This study was carried out on 22 Romanian Holstein cattle from experimental farm of National Research-Development Institute for Animal Biology and Nutrition. The cows were in the first lactation, kept in free stalls system and were milked twice daily. The average test day milk was 20.44l. The diet included corn silage, alfalfa hay and compound feeds (cereals, sunflower meal, soya meal and vitamin-mineral premix). For the determination of milk quality indices 2 sampling-day were performed in the middle of lactation from all 22 cows.

Determination of milk quality indices

The total percentage of milk solids non-fat (SNF), fat and protein content were determined using an Ekomilk M analyzer (Bultteh 2000 Ltd, EON Trading LLC, Delaware, USA).

Preparation of milk samples for total protein determination

15 ml milk from each sample was centrifuged at 5000 rpm at 4°C for 30 minutes in order to allow the fat to separate at the surface. The skimmed milk was preserved at -20°C until analysis.

Milk protein and determination separation

The Bradford method (Krauspe, 1986) was used to determine the concentration of total milk proteins before their separation. Briefly, 5 μ l of milk samples (diluted 1:40) were mixed with 250 μ l Bradford reagent in 96 wells plates (Corning, Sigma-Aldrich, Steinheim, Germany) and incubated at room temperature for 15 minutes. The absorbance was measured at 595 nm using a microplate reader (Sunrise™, Tecan Group Ltd, Mannedorf, Switzerland).

Milk proteins were further separated by SDS-PAGE electrophoresis (Mini-Protean 3 BioRad system, BioRad, Hercules CA, USA) in 15% polyacrylamide gel. 5 μ g of protein samples were migrated for 1h and 30 minutes, to facilitate separation of proteins, based on their apparent molecular weight. After migration, gels were stained by immersion in staining dye (Commassie Brilliant Blue 250 R, BioRad, Hercules CA, USA) for 15 minutes. The apparent molecular weights of analyzed proteins were established in relation with a protein standard (Precision Plus Protein Standards, BioRad, Hercules CA, USA).

Gel visualization

The gels were scanned and subjected to densitometry quantification using the GelQuant software (DNR Bio-Imaging Systems Ltd, Jerusalem, Israel). Precision Plus Protein Kaleidoscope Standard (Bio-Rad), containing known proteins with different molecular weight was used to determine the apparent molecular weights of protein fractions of interest. The relative expression of milk proteins was expressed as Arbitrary Units.

Statistical analysis

Student's test was used to analyze the differences between all samples. P values < 0.05 were considered significant. All data were expressed as mean \pm standard error of the mean (SEM).

Ethics statement

The research activities were performed in accordance with Directive 2010/63/EU of European Union for animal experimentation. Animals were cared in accordance with the Romanian Law 28/2011 for handling and protection of animals used for experimental purposes.

RESULTS AND DISCUSSION

Twenty-two milk samples from Romanian Holstein cattle were used in this study in order to determine several indices (quality parameters and

protein distribution) important for breeding process. The sampling day and milk chemical composition assays were performed during the milking period of the Romanian Holstein cattle (Table 1).

Our results showed that milk samples had a content of 4.39% fat and 3.38% total protein. Literature data show variations in milk composition for Holstein breed from different country around the world. Thus, our values were higher than those obtained by Nobrega and Langoni (2011) for Holstein cows from Brazil (3.25% fat and 3.01% protein). Yang et al., (2013) reported also percentages in Chinese Holstein cow's milk (3.91% fat and 3.10% protein) while lower percentage of fat and higher of protein (3.80% fat and 3.79% protein) was reported by Kuczynska (2012) in Polish Holstein Friesian.

Table 1. Indices of milk quality in Romanian Holstein cattle during milking period

Sampling day	Fat (g %)	Protein (g %)	SNF (g %)
	Mean \pm SEM	Mean \pm SEM	Mean \pm SEM
1	4.43 \pm 0.25	3.45 \pm 0.03	9.15 \pm 0.08
2	4.34 \pm 0.15	3.31 \pm 0.03	8.76 \pm 0.08
General mean	4.39 \pm 0.04	3.38 \pm 0.07	8.96 \pm 0.19

The identification and distribution of main milk proteins were further analyzed by SDS PAGE electrophoresis (Table 2). The following proteins were identified in milk sample from Romanian Holstein cattle: α 1-casein and α 2-casein, β -casein, κ -casein, α -lactalbumin (La) and β -lactoglobulin.

Table 2. Proportion of main protein fractions in milk of Romanian Holstein cattle

Protein fraction	% of total protein Mean \pm SEM
Total caseins	77.28\pm2.56
α 1-casein+ α 2-casein	36.01 \pm 1.46
β -casein	31.45 \pm 0.87
κ -casein	9.82 \pm 0.69
Total whey proteins	22.72\pm1.38
β -lactoglobulin	18.16 \pm 1.09
α -lactalbumin	4.56 \pm 0.40

*Protein fractions were separated by SDS-PAGE and intensity of bands was analyzed by densitometry using specialized software. The results were expressed as percentage from total milk protein analyzed by SDS-PAGE. The mean value \pm standard error of the mean (SEM) were calculated for each analyzed protein.

The analysis of the milk proteins (Table 2) showed that the caseins accounted 77.28% of the total milk proteins, while the major proteins from the whey represented 22.72% of the total protein. α 1-casein + α 2-casein had

a higher expression (36.01%) followed by β -casein (31.45%), β -lactoglobulin (18.16%), κ -casein (9.82%) and α -lactalbumin (4.56%) of the total protein.

The analysis of the casein fractions showed that α s1-casein + α s2-casein had the highest proportion, 46.59%, of all the caseins, followed by β -casein (40.70%) and κ -casein 12.71% (Table 3).

Table 3. Proportion of casein fractions in milk of Romanian Holstein cattle

Casein fraction	% of total casein Mean \pm SEM
α s1-casein+ α s2-casein	46.59 \pm 1.89
β -casein	40.70 \pm 1.13
κ -casein	12.71 \pm 0.89

Casein fractions were separated by SDS-PAGE and intensity of bands was analyzed by densitometry using specialized software. The results were expressed as percentage from total milk casein. The mean value \pm standard error of the mean (SEM) were calculated for each analyzed casein.

The average relative protein concentration of the major milk protein in Romanian Holstein was in the range of those reported for Holstein cattle breed. Results of other studies showed also a variation of casein fractions within the Holstein breed around the world. Genetic variation is a major factor contributing to milk protein variation, with 55% of the variation observed in milk composition explained by genetics, and the remaining 45% explained by other external factors (Oltenacu and Algers, 2005). The effect of seasonal variation on casein composition is relatively large, particularly the proportions of α s1-casein, α s2-casein and κ -casein within total casein. For example, the relative proportion of casein fractions on total milk protein in Italian Holstein population was 74.3%, 76.7 % and 76.5% in the summer, winter and spring (Bernabucci et al., 2015) lower than in Romanian Holstein (77.28%). With the exception of κ -casein, the proportion of α s1-casein, α s2-casein and β -casein showed the lowest value during summer and highest values in the winter (Barber 2007). By contrast, Buitenhuis et al., (2016) observed that the milk of Danish Holstein had a higher relative concentration of β -casein (36%) compared to the milk of Danish Jersey (28%) and with Romanian Holstein from our study (31.45%).

It has been observed that milk proteins differ from one population to another within the Holstein breed. For example, Maurmayr et al., (2018) observed in Italian Holstein cow's population a percentage of 81.71% caseins from total protein and 18.29% whey protein which were different than the values obtained by Bernabucci et al., 2015 in another population of Italian Holstein. In milk of Italian Holstein cows' population analyzed by Maurmayr et al., (2018) the caseins had the following proportions: 10.89% κ -casein, 33.11% β -casein, 29.27% α s1-casein, 8.44% α s2-casein while the whey

proteins registered 2.48% α -lactalbumin and 15.81% β -lactoglobulin. Schopen et al., (2009) found in the milk of Dutch Holstein- Friesians milk 75% caseins from total protein. The main caseins were α s1- casein and β -casein which made up 34 and 27% of the total protein. The whey protein (α -lactalbumin+ β -lactoglobulin) are present in low percentage representing only 10.79% from total protein. In our study the content of caseins was higher than in Dutch Holstein- Friesians. Franzoi et al., (2019) observed that the proportion of α -casein and β -lactoglobulin in Italian Holstein-Friesian breed (41.64% and 5,91% respectively) was lower compared with results of Schopen et al., 2009 in first-parity Dutch-Holstein-Friesian cows, whereas β -casein was higher compared with the same study (30.44% and 27.7%, respectively). Franzoi et al., 2019 considered that such difference can be attributed to the different cow parities and lactation stages including the sampling, to diversities in farming system and area.

Variation of protein fraction was determined by the effects of breed, parity, lactation stage and season. For instance, Kuczynska et al., (2012) observed variations of protein fraction when compared two cows breed, found higher content of α -lactalbumin and β -lactoglobulin in Polish Holstein Friesian compared to Montbeliarde cows.

Franzoi et al., 2019 observed that α -casein and k-casein increased in milk of older cows compared with first and second parity cows. Conversely, β -casein and α -lactalbumin decreased with parity order and β -lactoglobulin remained almost stable.

Protein fraction percentages showed variation across stage of lactation. Thus, α -casein decreased from 5 to 45 days in milk (DIM) and then slightly increased until 305 DIM and β -casein increased from 125 to 155 DIM and slightly decreased thereafter. A constant decrease of k-casein was observed through the entire lactation. The whey protein, α -lactalbumin and β -lactoglobulin decreased until 75 DIM and increased during the remaining part of lactation (Franzoi et al., 2019). In our study the caseins reached a maximum in mid lactation.

A distribution of milk samples related to the expression level (high, medium and low) of protein of interest was shown in Table 4.

In our study, percent analysis showed that the majority of milk samples was characterized by a medium expression level of both caseins and whey proteins (45.45-77.27%) followed by the higher level of expression (13.64-90.91%). There were statistically significant differences especially between cattle groups characterized by high and medium expression levels of all analyzed milk proteins. For α -lactalbumin expression, statistically significant difference between subgroups with high versus low expression level and medium versus low expression level were found.

Student's tests were used to analyze the difference between protein expression levels of all samples. P-values < 0.05 were considered significant (Table 5).

Table 4. Distribution of cattle groups depending on milk protein expression

Group	Protein	% of total samples
High(n=20)	β-casein	90.91
Medium (n=2)		9.09
Low (n=0)		0.0
High(n=4)	k-casein	18.18
Medium (n=17)		77.27
Low (n=1)		4.55
High (n=6)	α ₁ casein+	54.55
Medium (n=15)		45.45
Low (n=1)		0.00
High (n=6)	β-lactoglobulin	27.27
Medium (n=15)		68.18
Low (n=1)		4.55
High (n=6)	α-lactalbumin	13.64
Medium (n=15)		63.64
Low (n=1)		22.73

Table 5. Evaluation of statistic differences between the distribution of cattle groups depending on milk protein expression

Cattle groups	T-test(p value)				
	α ₁ -casein + α ₂ -casein	β-casein	k-casein	β-lg	α-la
High vs Medium	<0.001	0.008	<0.001	<0.001	0.018
High vs Low					0.004
Medium vs Low					<0.001

The heritability for the relative protein concentration obtained by Schopen et al., (2009) was high and ranged from 0.25 for beta-casein to 0.80 for beta-lactoglobulin in Dutch Holstein Friesians. The heritability for summed whey fraction (0.71) was higher than that for the summed casein fraction (0.4). High heritability estimates were found by Buitenhuis et al., (2016) in Danish Holstein for protein percentage (0.4), casein percentage (0.43), k-casein (0.77), beta-lactoglobulin (0.58) and alfa-lactalbumin (0.40). The

studies suggested that there were opportunities to change the milk protein composition in the cow's milk using selective breeding.

CONCLUSION

In our study the percentage of fat milk is higher than other studies in different Holstein cattle populations and the percentage of protein milk ranged in the values from literature.

Variation of protein fraction was determined by the effects of parity, lactation stage and season in Holstein populations.

The Romanian Holstein cows had different proportions of casein and whey proteins, α s1-casein + α s2-casein had a higher expression (36.01%) followed by β -casein (31.45%), β -lactoglobulin (18.16%), κ -casein (9.82%) and α -lactalbumin (4.56%). Selective breeding could change milk protein composition to improve milk quality and milk coagulation properties for Romanian Holstein cows.

ACKNOWLEDGEMENTS

This work was supported by funds from the Nucleus Program, of the Romanian Ministry of Research and Innovation, project number PN 16410602.

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