MILK COMPOSITION AND IDENTIFICATION OF PROTEIN POLYMORPHISM IN MONTBÉLIARDE COWS TO SELECT THE BEST VARIANTS AND IMPROVE CHESSE QUALITY

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Abstract

The main purpose of this study was to identify the proteins polymorphism in Montbéliarde cows to discover and select the best individuals whit casein variants suitable for a very good ability of making cheese. Milk composition was analysed for fat (3.83%) and protein (3.38%). Fat percentage manifested a high variability and ranged between 6.06% and 1.21%, while milk protein didn't have the same variation with closed limits (3.89 and 3.28%). SDS-PAGE analysis revealed, after electrophoresis, six protein fractions in milk samples: αs1, αs2, β, k-CN, and β-LG, and α-LA. By densitometry analysis, three levels of expressions of the same type of protein fraction were discovered in each milk sample. The casein group, composed by four fractions αs1-, αs2-, β- and k-casein (32.8%, 7.3%, 34.3%, 17.6%) show in fact that they are 92% of the total milk protein and 8% are albumins similar with other studies for cows. First protein fraction αs1-casein, was with medium expression manifested by 54.54% of the cows, followed by that one with strong (31.82%) and low expression (13.64%). On the third place in milk composition is situated β-casein with strong expression for 4.54% of the cows. Medium expression level of the β-casein was manifested by the majority of the analysed Montbéliarde cows (72.73%) and 22.73% of it had low expression level, while only 4.54% of them have recorded strong expression level. Further investigations will be necessary to confirm the results by PCR-RFLP analysis to identify Montbéliarde cows caseins polymorphism with the genetic variants for each protein locus. This will allow keeping the most suitable animals with strong genetic expression and high content in caseins very well correlated with milk.

Key words: Montbéliarde, dairy cows, milk composition, milk proteins polymorphism

INTRODUCTION

Milk production has a major importance in cheese industry because is the main material for yogurt, kefir, cheese, products with very good qualities for human consumption. Cow milk is preponderant on cheese production globally and is an important nutrient used daily in human diets. Especially, cow milk composition feat perfectly, because it has many qualities with a great impact in health people, mostly for a very good start of the children and with benefits for adults and also for the age people. Dairy cows, in present days are characterised by moderate performances having good functional traits like longevity with importance in productive life, fertility, health and also with outstanding quality in milk composition, fat, protein, lactose, vitamins. Dairy cows breeders knows that have to improve and to maintain these traits. The main purpose of the present study was to identify milk protein fractions in Montbéliarde cows, which are originated from France, with very good performances in milk production. Body conformation of Montbéliarde cows is built for milk and meat production, with docile behaviour, very well adapted to natural and artificial environment with good health and robustness. There are studies where Montbéliarde cow were compared with other diary breeds like Holstein Friesian and had a better casein index, with the capacity to transform casein fraction in a higher amount of cheese, with great milk protein and fat content, [2]. With a

good cheese making ability was remarked also Russian Black-Pied cows who hadwithκ-casein fraction, variant *BB*very good results, with higher content of fat and protein recoveries in cheese, [1]. The same results were obtained also, [11] in Slovak Pied breed, in this case investigating the influence of κ- casein and β-lactoglobulin genotype on milk composition and its technological properties by combining these two protein fractions. Another dairy cow breeds were investigated also, Chinese Holstein, Jersey and buffalo for the variants of κ-casein who affect casein content, protein content and cheese yield, as well as curd firmness, and was observed that β-lactoglobulin is significantly associated with fat, protein, casein, total solid content and cheese yield [14, 5,8].The purpose of this study was determine milk composition and to investigate and identify the protein polymorphism in Montbéliarde cows to establish who are the best variants correlated with milk fat and protein content.

MATERIAL AND METHOD

Animals Milk samples from 22Montbéliardecowsfrom INCDBNA Balotesti research farm, Ilfov County were used to determine milk composition and protein polymorphism.

Sample preparation for total milk protein polymorphism determination: 50 ml milk from each sample were centrifuged at 5000 rpm at a temperature of 4°C for 30 minutes in order to allow fat milk to be separated at the surface. The skimmed milk was preserved at -20°C until analysis. *Protein determination*: milk total proteins were determined using Bradford method [10]. Skimmed milk samples were diluted 1:40 using plates with 96 wells (Corning, Sigma, Redox, Romania) and used to determine the total milk protein. The wells were filled with 5µl sample and 250 µl Bradford reagent. The samples were incubated at room temperature 45 minutes. The absorbance was measured at 595 nm using a microplate reader (TECAN SUNRISE, Austria) compared to blank.

Milk composition was determined with Ekomilk M analyser (Bulteh 2000 Ltd, EON Trading LLC, USA).

Milk protein electrophoresis protein polymorphism were determined by vertical polyacrylamide gel electrophoresis (Mini-Protean 3 BioRad system, Romania) using migration gel with 15% polyacrylamide and concentration gel with 5% polyacrylamide, migration time 1 h and 30 minutes at 200 V. Precision Plus Protein standards ladder contains ten recombinant protein bands that were used as reference for milk caseins and albumins which are migrating between 25 and 19 kDa (beta-CN 24 kDa, kappa-CN 19-20 kDa, alfa-CN 23-25 kDa). After protein migration, the gels were stained by immersion in dye solution (Coomassi Blue 250 R BioRad, Romania) for 45 minutes and were decolouredby immersion in a solution with 10% acetic acid and 2.5% methanol. *Gel visualisation* was done with transiluminator Benchtop 2UV and analysed with Image J software.

RESULTS AND DISCUSSION

Table 1 shows that the fat percentage of the milk samples ranged between 3.83 % and 1.21% very high variability between all cows. Protein percentage has no high variability and ranged between 3.38% and 3.28% like in other cow breeds cited in scientific literature. Regarding the fat content a higher percentage was found in Montbéliarde cows by Beata in comparison with fat percentage obtained in the present research (3.83%). In this case total protein percentage (3.77%) was almost similar with that obtained in the present study (3.38%).

Table 1 Milk composition in Montbéliarde cows

	Fat (%)	SNF (%)	Protein (%)
Mean	3.83	8.95	3.38
Standard deviation	1.34	0.76	0.29
Error	0.29	0.16	0.06
Variability coefficient	34.93	8.45	8.49
Maxim	6.06	10.3	3.89
Minim	1.21	8.75	3.28

The SDS-PAGE analysis revealed after electrophoresis six protein fractions in milk samples from Montbéliarde cows (figure 1). These proteins are α s1-casein, αs2-casein β-casein, k-casein, β-lactoglobulin, and α-lactalbumin. The casein group composed by four fractionsαs1-casein, αs2-casein β-casein and k-casein (32.8 %, 7.3%, 34.3%, 17.6%) show in fact that they are 92% of the total milk protein and 8% are albumins similar with other studies for cows.
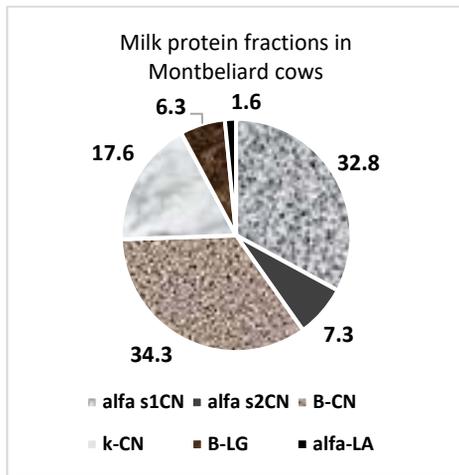

Fig. 1 Milk protein fractions in Montbéliarde cows

Using densitometry analysis we determined the different expression of the same type of protein fraction for each cow investigated (Tables 2, 3; Figures 2, 3). The purpose of this study was to quantify the expression of milk protein fractions which are with strong, medium and low expression. Montbéliarde cows investigated in this study had32.8% of the total milk protein expressed by αs1-casein fraction in the limits known compared with that obtained in similar research on genetic variability of cows. Theαs1-casein family constitutes up to 40% of protein fraction in bovine milk [7].

In the present research was found six protein fractions (α s1-casein, α s2-casein β-casein, k-casein, β-lactoglobulin, and α-lactalbumin) with different level of expression in milk composition. First protein fraction αs1-casein, was with medium expression manifested by 54.54% of the cows, followed by that one with strong expression of 31.82% and with low expression of 13.64%.Especially genotype *BB*of the αs1-caseinis associated with a higher milk quantity,[12]. The second protein fraction investigated in the present study was αs2-casein, which had recorded 7.3 % from the total milk protein of the Montbéliarde cows, with 2.7 % lower than that obtained in other similar studies where αs2-casein constitute up to 10% of the casein fraction in bovine milk, [7]. In the present case Montbéliarde cows expressed two levels for milk proteins with strong expression manifested by 4.54% of the individuals and with low expression for 9.09% of it, while the most of them didn`t expressed this type (s2-casein) of milk protein fraction (86.37%). On the third place in milk composition for protein is situated β-casein with strong expression level recorded by 4.54% of the cows. Medium expression level of the β-casein was manifested by the majority of the analysed Montbéliarde cows (72.73%) and 22.73% of it had low expression level, while only 4.54% of them have recorded strong expression level, (Table 4). There are similar studies for milk proteins who show that 45% of the casein are constitutes by β-casein fraction in bovine milk, [7]. Similar research was done to establish if there is associations between alleles found in β-casein locus and some milk production traits milk yield and fat, protein, casein and lactose content in a Holstein-Friesian population reared in Romania, and shows that A2B genotype has slightly influence on the casein content (A2B > A1A1 > A1A2 > A2A2 > A1B), although the differences between *CSN2* genotypes for this trait were not significant [4]. Three expression levels were observed in Montbéliarde cows for k-casein milk fraction with medium value for 54.54% of the individuals, followed by low expression for 40.92% of the cows and strong expression manifested only in 4.54% of them. Similar research were done to found the association between milk protein fractions with milk quantity and fat percentage in Chinese Holstein cows and two genotypes of k-casein were found *AA* and *AB,* which are responsible with a higher fat percentage [15].

Tow albumin protein fractions were analysed in Montbéliarde cows, β-lactoglobulin and α-lactalbumin. β-lactoglobulin manifested strong expression in 9.09% of the investigated cows and 90.91% were with low level for protein expression (table 4). There are many researches that have similar subject to identify the presents of β-lactoglobulin in Turkish cattle breeds, with suitable milk composition for cheese, [6]. Also, β-lactoglobulin is associated with yogurt qualities [9]. There are studies that want to clear the situation in case of Simmental and Brown cattle, regarding the association of milk proteins with a higher content in fat correlated with β-lactoglobulin *BB* [3].

The second protein fraction observed in Montbéliarde cows in the present study was α-lactalbumin with three level of milk protein expression. Strong protein expression of α-lactoglobulin was manifested by 4.54% of the investigated cows, while 13.64% of them recorded medium expression. Beata et al., 2012, [2], in his research made a comparison between Montbéliarde and Holsteine-Friesian cows regarding protein fractions and found a higher content of α-lactalbumin and β-lactoglobuline for Holstein-Friesian (table 4). Figures 4, 5 and 6 show milk proteins bands migrated in SDS-PAGE vertical gel. In each polyacrylamide gel, first well is for the marker with ten recombinant proteins as reference for caseins and albumins followed by the milk samples.

Table 2 Milk proteins densitometry of the Montbéliarde cows (samples 1-11)

	1	2	3	4	5	6	7	8	9	10	11
α s1CN	30412	33305	35098	30803	27779	34427	36728	38101	36681	27879	28474
α s2CN					10937						3325
β-CN	30540	36067	34189	33826	34623	36753	36701	37319	33307	28542	31394
k-CN	19874	14169	21545	21221	4637	19139	16523	25895	18290	13781	17582
β-LG	3519	5309	3753	4132	20277	6104	4888	5371	2866	2323	5685
α -LA	438	147	623	492	7449		497	955	663	484	3404

Table 3 Milk proteins densitometry of the Montbéliarde cows (samples 11-22)

	12	13	14	15	16	17	18	19	20	21	22
α s1CN	28561	31919	30562	34165	35841	5949	29871	26948	28853	26966	35702
α s2CN	6223										
β-CN	31558	28927	33225	31612	34340	5814	32561	25238	32641	29899	47224
k-CN	4371	16734	20312	17632	14983	2674	18344	15375	19879	17772	21590
β-LG	19761	3554	2579	3404	5877	8942	4377	2654	6338	3149	5567
α -LA	2625		630	204		1868	897	1259	2062	1756	1202

Table 4 Protein expression in Montbéliarde cows

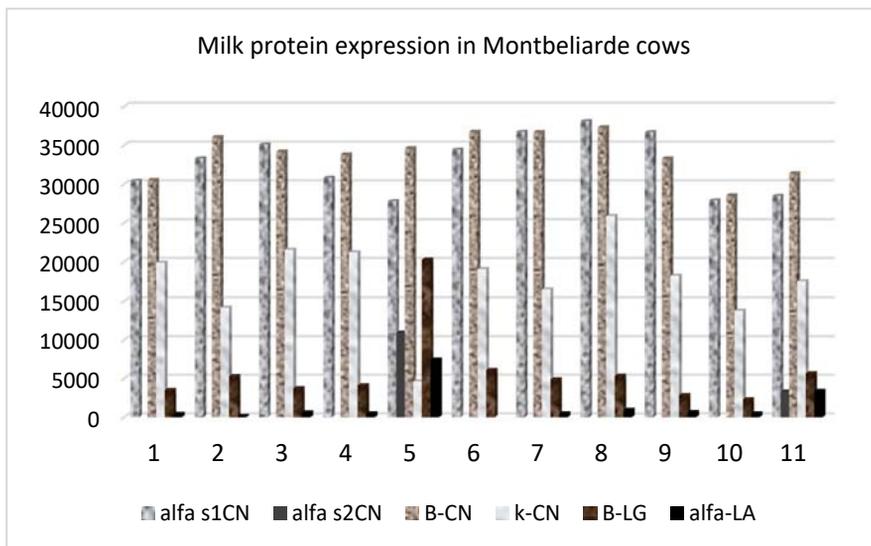| Protein fractions	Level of protein expression			
	Simple expression (%)	Medium expression (%)	Strong expression (%)	No expression (%)
α s1CN	13.64	54.54	31.82	-
α s2CN	9.09	-	4.54	86.37
β-CN	22.73	72.73	4.54	-
k-CN	40.92	54.54	4.54	-
β-LG	90.91	-	9.09	-
α -LA	81.82	13.64	4.54	-

Fig. 2 Milk proteins expression in Montbéliarde cows sample 1-11
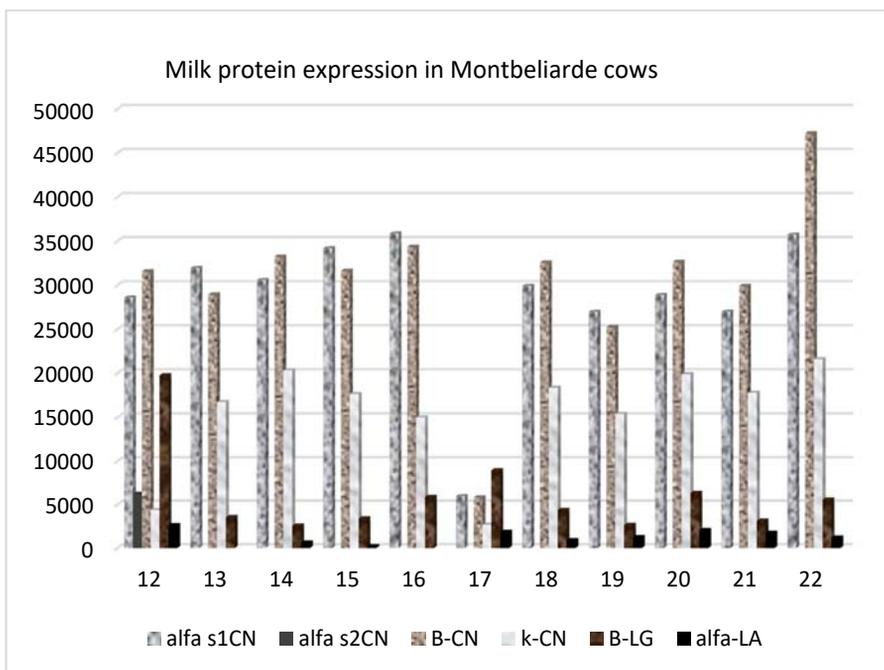

Fig. 3 Milk proteins expression in Montbéliarde cows sample 12-22
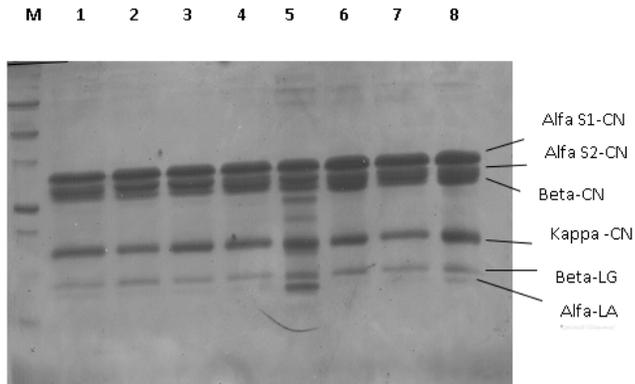
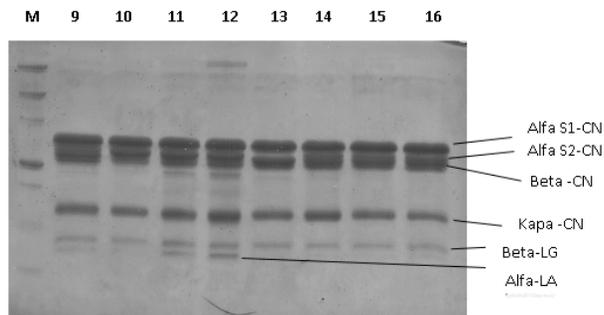Fig. 4 Milk proteins expression in Montbéliarde cows sample 1-8

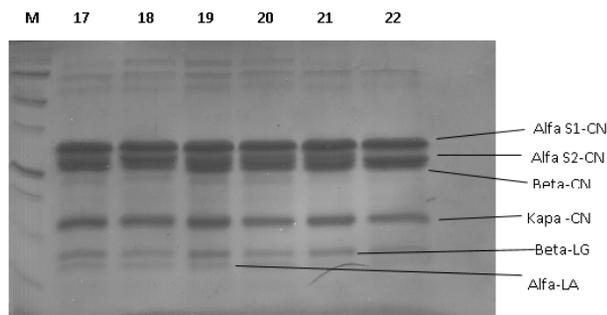Fig. 5 Milk proteins expression in Montbéliarde cows sample 9-16

Fig. 6 Milk proteins expression in Montbéliarde cows sample 17-22

CONCLUSIONS

Especially, cow milk composition feat perfectly, because it has many qualities with a great impact in health people, mostly for a very good start of the children and with benefits for adults and also for the age people.

Montbéliarde cows investigated in the present study manifested a very strong expression of the αs1-casein, (31.82%) which it is very well correlated with milk quantity. On the second place with strong expression is situated β-lactoglobulin (9.09%), a milk protein fraction associated with a higher content in fat. Than in the third place, with strong expression are situated αs1-casein, β-casein, k-casein and α-lactalbummin

(4.54%). Further investigations will be necessary to confirm the results by PCR-RFLP analysis to identify the genotypes for each proteins locus in Montbéliarde cows.

By identification of these animals, with strong genetic expression and high content in caseins could be improved the ability to making cheese, very well correlated with milk quantity and quality.

ACKNOWLEDGEMENTS

REFERENCES

[1] Alipanah M., Kalashnikova L. A. 2007: Influence of K-Casein Genetic Variant on Cheese Making Ability, Journal of Animal and Veterinary Advances, 6, (7), 855-857.

[2] Beata K., Puppel K., Golebiewski M., Kordyasz M., Grodzki H., Brzozowski P. 2012: Comparison of fat and protein fractions of milk constituents in Montbéliarde and Polish Holstine-Friesian coes from one farm in Poland, ACTA VET. BRNO, 81, p.139-144.

[3] Buchberger J., Dovc P. 2000: Lactoprotein Genetic Variants in Cattle and Cheese Making Ability, Food technology biotechnology, 38, p. 91-98.

[4] Bugeac T., Dumitraş C. M., Creangă Şt. 2015: Genetic Polymorphism of beta-Casein Gene and its Associations with Milk Traits in Holstein Friesian Cows, Scientific Papers: Animal Science and Biotechnologies, 48, (1), p. 103-107.

[5] Celik S. 2003: beta lactoglobulin genetic variants in Brown Swiss breed and its association with compositional properties and rennet clotting time of milk, International Dairy Journal, 13, p. 727-731.

[6] Dinc H., Ozkan E., Koban E., Togan I. 2013: Beta-casein A1/A2, kappa-casein and beta-lactoglobulin polymorphism in Turkish cattle breeds, Archiv Tierzucht, 56, 65, p. 650-657.

[7] Farrell H. M., Jimenes-Flores R., Bleck G. T., Brown M. E., Butler J. E. 2004: Nomenclature of the Proteins of Cows' Milk-Sixth Revision, Journal Diary Science, 87, 1641-1674.

[8] Hallen E., Wedholm A., Andren A., Lunden A. 2008: Effect of beta-casein and beta-lactoglobulin genotypes on concentration of milk protein variants, Jornal Animal breeding and Genetics, 125, p. 119-129.

[9] Hallen E., Allmere T., Lunden A., Andren A. 2009: Effect of genetic polymorphism of milk proteins on rheology of acid-induced milk gels, International Dairy Journal, 19, p. 399-404

[10] Krauspe R., Scheera A. 1986: Coomassie brilliant blue G-250 dye – binding technique for determination of autolytic protein breakdown in Euglena gracilis and comparition to other methods of autolysis measurement, Anal Biochem, 153 (2), 242-250.

[11] Michalcova A., Krupova Z. 2007: Influence of composite k-casein and β-lactoglobulin genotypes on composition, rennet ability and heat stability of milk of cows of Slovak Pied breed, Czech Journal of Animal Science, 52, (9), p. 292-298.

[12] Ng-Kwai-Hang, Hayes K.F., Moxley J.F., Monardes J.E., H.G., Relationships between milk proteins polymorphisms and major milk constituents in Holstein-Friesian cows, J. Dairy Sci., 1986, doi.org/10.3168/jds.S0022 0302(86)80364-2.

[13] Pelmus R. S., Pistol G.C., Lazar C., Marin D.E., Gras M., Radu M., Ghita E. 2012: preliminary study on milk composition and protein polymorphism in Romanian local sheep breed Teleorman Black Head Tsigai, Romanian Biotechnological letters, vol.17, no. 5, p. 7582-7591.

[14] Ren D. X., Miao S. Y., Chen C. X., Liang X. W., Liu J. X. 2011: Genotyping of the k-casein and β-lactoglobulin gene in Chinese Holstine, Jersey and water buffalo by PCR-RFLP, Journal of Genetics, 90, 1, p.e1-e5http://wwwias.a.in/jgenet/OnlineResources/90/el.pdf]

[15] Zhang R. F., Chen H., Lei C. Z., Fang X. T., Zhang Y. D., Hu S. R., Su L. H. 2007: Association between PCR-RFLP Polymorphisms of Five Gene Loci and Milk Traits in Chinese Holstein, Asian Australian Journal Animal Science, 20, 2, p. 166-171