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PhD THESIS ABSTRACT

The PhD thesis entitled “*Genetic polymorphism of milk proteins and the relationships between their genetic variants with quantitative and qualitative traits in some cattle populations*“ it is divided in two parts: the bibliographic study and the own research, adding introduction, conclusions and recommendations and bibliography.

The bibliographic study comprises four chapters:

❖ *Chapter I. This chapter presents the current situation of the cattle and milk production globally and nationally, as well the trends and statistics according to Food and Agriculture Organization of the United Nations (FAO), Department of Agriculture the United States (USDA) and the National Institute of Statistics (INS).*

❖ *Chapter II. This chapter presents the structure and chemical composition of cow milk and compared to other species of livestock, and the structure and function of the concentration of the major protein of cow's milk.*

❖ *Chapter III. This chapter describes some of the molecular techniques used to identify genetic variants of milk proteins as the major gene expression and DNA level.*

❖ *Chapter IV. Includes existing data from the literature concerning the production and quality of milk, genetic parameters of quantitative and qualitative characteristics of milk, genetic polymorphism of the major milk proteins, the structure of genetic loci major milk proteins and relationships between genetic variants and the milk qualitative and quantitative traits in Holstein-Friesian, Montbéliarde and Romanian black spotted (BNR) cattle breeds.*

The original research it is divided into six chapters:

❖ *Chapter V. In this chapter we present purposes, the experimental protocol, namely the importance of research and institutional framework in which the research took place.*

The research was conducted in several units, or specialized institutions: Chilia dairy farm; Giani dairy farm; Research and Development Center for Bovine Raising – Dancu Iași; Faculty of



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Animal Science USAMV Iași; University of Agricultural Sciences and Veterinary Medicine "Ion Ionescu de la Brad – Iași"; Institute of Life Sciences at the Veterinary Medicine Cluj-Napoca; Foundation for Milk Quality Control, Floresti - Cluj-Napoca and the Department of Agricultural and Environmental Sciences of the University of Udine - Italy.

❖ *Chapter VI.* This chapter describes the research methods and material used in order to achieve the research objectives. The research was conducted in three populations of cattle from three breeds: Holstein-Friesian, Montbéliarde and Romanian black spotted (BNR). In order to perform physical and chemical analyses, milk samples were collected seasonally - in accordance with the current regulations. We used Infra Red Spectroscopy (MIR). Data about milk production and lactation rank were taken from the existing farms databases. For molecular genetics analyses, milk samples were collected once from each animal - respecting the existing methodology of milk sampling. We used the isoelectric focusing electrophoresis (IEF). Statistical analysis of the results was performed using SPSS v.19 for Windows, Microsoft Excel, Microsoft Office and Windows SAVC. For genetic parameters estimating, as well for variance and covariance we used Restricted Maximum Likelihood mathematical model (REML) based on the animal pedigrees. To assess the state of genetic equilibrium in populations according to Hardy-Weinberg theory, we used chi square test (χ^2).

❖ *Chapter VII.* In this chapter the results of their own research on productive performance of cattle breeds studied. Mean and variability estimators for the characters on the production and quality of milk were calculated, as well the differences between means for each character in each population. Thus, for the fat content the differences were statistically significant ($p < 0.001$) between the three breeds for all seasons.

For milk protein content there were significant differences statistically ($p < 0.001$) between Holstein-Friesian and Montbéliarde breeds for the winter season, with a superior mean for Holstein-Friesian breed. In the spring season, both Montbéliarde and Holstein populations had similar average values for the protein percent. There were statistically significant differences ($p < 0.001$) between Holstein and BNR, respectively between Montbéliarde and BNR.

For summer and autumn seasons there were statistically significant differences ($p < 0.001$) between the three populations. Holstein-Friesian breed had the highest mean for fat percent in both the summer and autumn.

Regarding the milk content in casein for winter sampled milk, the statistically differences were highly significant ($p < 0,001$) between Holstein-Friesian and Montbéliarde, respectively between BNR and Montbéliarde, with a top average for Holstein-Friesian breed, followed by BNR breed.



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For the casein content in the milk collected in spring the differences were statistically significant ($p < 0.05$) between Holstein-Friesian and Montbéliarde, respectively between Holstein-Friesian and BNR. Between BNR and Montbéliarde the differences were statistically highly significant ($p < 0.001$) for the same trait. For milk samples collected in summer, the differences were statistically highly significant ($p < 0.001$) between Holstein-Friesian and Montbéliarde. For the milk samples collected in autumn, statistically highly significant differences ($p < 0.001$) were recorded between Holstein-Friesian and BNR. The Holstein-Friesian breed had the highest average value in all seasons for this trait.

For somatic cell count the differences were statistically highly significant ($p < 0.001$) between Holstein-Friesian and Montbéliarde, as well between Montbéliarde and BNR in winter, spring and summer. In all three seasons Montbéliarde breed recorded the highest values, despite the fact that it is characterized as having a low incidence of mastitis and high resistance to diseases.

❖ *Chapter VIII.* This chapter presents the genetic structure for the six loci of major milk proteins in the studied cattle populations. The individuals from Holstein-Friesian and Montbéliarde populations were genotyped at the following loci: CSN1S1 (encoding α_{S1} -CN) CSN2 (encoding β -CN) CSN1S2 (encoding α_{S2} -CN) CSN3 (encoding K-CN), LGB (encoding β -LG) and (encoding α -LA).

Genetic variations occurring in the milk protein gene can be controlled as homozygote genotypes AA and BB type and heterozygous AB type based on the DNA sequence. At CSN1S1 locus we identified two alleles α_{S1} -CN*B and α_{S1} -CN*C. The dominant genotype is homozygous BB, due to high frequency of allele α_{S1} -CN*B. α_{S1} -CN*C allele is very rare, therefore presents very low frequencies for the two populations. Allele C was not found in the Montbéliarde population. Because of the high frequency of α_{S1} -CN*B allele, the dominant genotype in the two populations is α_{S1} -CN BB with its frequency over 94%. Both analyzed populations are in genetic equilibrium according to Hardy-Weinberg principle, as there were no statistically significant differences ($p > 0.05$) between the observed and expected frequencies.

At the CSN2 locus 7 genotypes were identified. These genotypes are formed from A_1 , A_2 , B and C alleles combination. A_1A_1 și A_2C genotypes were identified only in Holstein-Friesian population. In this population the dominant genotypes were A_1A_2 și A_2A_2 – both with the same frequency. The rarest ones were A_2C and BB genotypes. The A_2 allele had the highest frequency in both populations, this allele being the most common in *Bos* genus. On the other side, the β -CN*C allele had an extremely low frequency, almost insignificant in Holstein-Friesian population, while



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in the Montbéliarde population it was inexistent. In Montbéliarde population the A₂B genotype had the highest frequency, followed by A₂A₂ homozygous genotype.

The β-CN*A₁ allele had a higher frequency in Holstein-Friesian population, while the β-CN*B allele it was less common and had the highest frequency in Montbéliarde population.

Both Holstein-Friesian and Montbéliarde population were in genetic equilibrium according to Hardy-Weinberg principle, as there were no statistically significant differences ($p > 0.05$) between observed and expected frequencies.

At the α-LA locus we identified only the α-LA *B allele, which had a frequency equal to 1.

At LGB locus 2 genetic variants were identified: β-LG*A and B forming 2 homozygous genotypes AA and BB and a heterozygous one - AB. The β-LG*B allele had the highest frequency for both analysed populations. In Holstein-Friesian population the dominant genotype is β-LG AB with a frequency of 0,44 while in Montbéliarde population– the homozygous genotype β-LG BB with a frequency of 0,62. The β-LG*A allele had a higher frequency in Holstein-Friesian, while the β-LG*B allele had a higher frequency in Montbéliarde.

Both Holstein-Friesian and Montbéliarde were in genetic equilibrium according to Hardy-Weinberg law, because there were no statistically significant differences ($p > 0.05$) between the expected and observed frequencies.

❖ *Chapter IX.* In this chapter we present the results regarding the genetic parameters estimated for the studied populations. For milk production, heritability coefficients were 0.23 for Holstein-Friesian, respectively 0.22 for Montbéliarde. Genetic determinism for fat content is high for both populations, but higher ($h^2 = 0.67$) for Montbéliarde compared to Holstein-Friesian (0.64). For protein content were estimated very high heritability coefficients for both populations, and this is in agreement with results of other studies the specialty made on the two populations. Genetic determinism estimated for milk content in casein it is superior to Montbéliarde (0.29) compared to Holstein-Friesian (0.27). Both populations have a weak heritability coefficient for this trait. Heritability coefficient values for somatic cell count (SCC) estimated in our research it is similar for the two populations: $h^2 = 0.16$ for Holstein-Friesian and $h^2 = 0.14$ for Montbéliarde and indicates a low genetic determinism. In order to determine the interdependence level between the traits, we calculated phenotypic and genetic correlation coefficients for milk yield, fat content, protein content, casein content, the content of lactose and the somatic cell count (SCC / mL x1000) as well as protein fractions (α_{S1}-casein, β-CN,-CN K and β-LG).

❖ *Chapter X.* In this chapter we present the relationships between the identified genotypes identified at the polymorphic loci CSN1S1, CSN2, CSN3 respectively LGB and milk production, fat content, protein content, casein content, lactose content and milk somatic cell count (SCC) for Holstein-Friesian, Montbéliarde and Romanian black spotted (BNR).



Holstein-Friesian breed

CSN1S1 Locus

- *Milk yield*: BB>BC. Individuals homozygous for allele B had a higher milk yield compared to individuals with heterozygous genotype BC. There were no statistically significant differences ($p>0.05$) between the genotypes for this trait.
- *Fat content*: BB>BC. Cows with homozygous genotype α_{S1} -CN BB had a higher fat percent 0,8% (relative value) compared to cows with heterozygous genotype BC. There were no statistically significant differences ($p>0.05$) between the genotypes for this trait.
- *Protein content*: BC>BB. Milk obtained from heterozygous for C allele had a 0,2% higher content (relative value) higher compared with the milk produced by cows with BB genotype. There were no statistically significant differences ($p>0.05$) between the genotypes for this trait.
- *Casein content*: BC>BB. There were no statistically significant differences ($p>0.05$) between the genotypes for this trait.
- *Somatic cell score (SCC)*: BB<BC. We found approximately 164% (relative value) more somatic cells in the milk collected from cows with α_{S1} -CN BC genotype compared with the milk obtained from cows with α_{S1} -CN BB genotype. There were no statistically significant differences ($p>0.05$) between the genotypes for this trait.

CSN2 Locus

- *Milk yield*: $A_2A_2>A_1A_2>A_1B>A_2B>A_1A_1$. Statistically highly significant differences ($p<0.001$) were recorded between genotypes β -CN A_2A_2 and A_1A_1 .
- *Fat content*: $A_1A_2>A_2B>A_1A_1>A_2A_2>A_1B$. Statistically highly significant differences ($p<0.001$) were recorded between β -CN A_1A_2 and β -CN A_2A_2 și A_1A_2 genotypes.
- *Protein content*: $A_2B>A_1A_1>A_1A_2>A_2A_2>A_1B$. Statistically significant differences ($p<0.05$) were recorded between β -CN A_2B and A_2A_2
- *Casein content*: $A_2B>A_1A_1>A_1A_2>A_2A_2>A_1B$. There were no statistically significant differences ($p>0.05$) between the genotypes for this trait.
- *Lactose content*. Statistically distinct significant differences ($p<0.01$) were recorded between β -CN BB and A_1A_2 , respectively between β -CN BB and A_1B .
- *SCC*. The milk obtained from cows with A_1A_1 genotype had the highest SCC /mL, with 25,9% (relative value) higher than the milk collected from cows with A_1A_2 genotype, respectively with 26,9% (relative value) higher than the milk produced by cows with A_2B genotype. Statistically highly significant differences ($p<0.001$) were calculated between β -CN A_1B and A_2B .



CSN3 Locus

- *Milk yield.* Cows with heterozygous genotype AB produced with 733 kg more compared to homozygous AA ($p < 0.001$) and with 2555 kg more compared to cows with BB genotype ($p < 0.001$). The differences were statistically highly significant in both cases.
- *Fat content.* Homozygous individuals for K-CN*B allele produced a milk with higher fat content (with 1.6% - relative value) compared to AB heterozygous, respectively (2,6% more – relative value) compared to homozygous cows for K-CN*A allele. Although, there were no statistically significant differences ($p > 0.05$) between the genotypes for this trait.
- *Protein content.* Cows with K-CN BB genotype produced a milk with higher protein content compared to cows with AB and AA genotypes. Statistically highly significant differences ($p < 0.001$) were recorded between K-CN BB and AA, respectively between K-CN BB and AB genotypes.
- *Casein content.* Homozygous individuals K-CN BB produced a milk with 7% (relative value) higher casein content compared homozygous K-CN AA, respectively with 3,1% (relative values) compared to heterozygous K-CN AB. There were statistically distinct significant differences ($p < 0.01$) between K-CN BB and AA, respectively highly significant differences ($p < 0.001$) between K-CN BB and AB genotypes.
- *Lactose content.* The K-CN BB genotype had a positive influence on the lactose content of milk.. There were statistically distinct significant differences ($p < 0.01$) between K-CN BB and AA genotypes and highly significant differences ($p < 0.001$) between K-CN BB and AB genotypes
- *SCC.* Milk collected from cows with heterozygous genotype BB had the lowest SCC, with approximately 37.5% (relative value) lower compared with milk produced by cows with homozygous AA genotype. Statistically highly significant differences ($p < 0.001$) were recorded between K-CN AB and AA genotypes.

LGB Locus.

- *Milk yield.* Cows with heterozygous genotype β -LG AB produced with 780 kg more milk compared to cows with β -LG AA and with 940 kg more than the homozygous β -LG BB.
There were statistically distinct significant differences ($p < 0.01$) between β -LG BB and AB genotypes.
- *Fat content.* Homozygous individuals β -LG BB produced a milk with higher fat content compared to heterozygous individuals β -LG AB, respectively to homozygous ones β -LG AA. The statistically differences were highly significant ($p < 0.001$) in both cases.
- *Protein content* was higher in the milk sampled from cows with β -LG BB genotype compared with β -LG AA and AB. The differences were not statistically significant ($p > 0.05$).



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- *Casein content.* No statistically significant differences ($p > 0.05$) recorded between genotypes for this trait.
- *Lactose content.* Statistically significant differences ($p < 0.05$) recorded between β -LG BB and AB genotypes, while highly significant differences ($p < 0.001$) recorded between β -LG AB and AA genotypes.
- *SCC.* The milk sampled from cows with homozygous genotype AA had the lowest SCC /mL. Statistically significant differences ($p < 0.05$) recorded between β -LG AA and BB, respectively between β -LG AA and AB.

Montbéliarde breed

CSN1S1 Locus

In Montbéliarde breed for α_{S1} -CN locus we didn't identify genetic polymorphism, but for a single genotype formed by α_{S1} -CN*B allele in double dose.

CSN2 Locus

- *Milk yield:* β -CN*A₂A₂>A₁A₂>A₂B>A₁B. Statistically highly significant differences ($p < 0.001$) recorded between β -CN A₂A₂ and A₁B genotypes, respectively between β -CN A₂B and A₁B.
- *Fat content:* A₁A₂>A₂A₂>A₂B>A₁B. Statistically highly significant differences ($p < 0.001$) recorded between β -CN A₂B and A₁B.
- *Protein content.* Combination of β -CN*A₁ and A₂ alleles it is favourable in order to obtain a higher protein content of milk. Statistically highly significant differences ($p < 0.001$) recorded between β -CN A₁A₂ and A₂B genotypes.
- *Casein content:* A₁A₂>A₂B>A₂A₂>A₁B. Statistically distinct significant differences ($p < 0.01$) recorded between β -CN A₁A₂ and A₂A₂ genotypes.
- *Lactose content.* Milk gathered from cows with β -CN A₁B and A₂B genotypes had the highest lactose content. Statistically highly significant differences ($p < 0.001$) recorded between β -CN A₂B and A₁A₂ genotype.
- *SCC.* Milk produced by cows with A₁B genotype had the lowest SCC /mL. Statistically highly significant differences ($p < 0.001$) recorded between β -CN A₁A₂ and A₂A₂ genotypes, between A₂B and A₁B genotypes, respectively between A₁B and A₂A₂ and between β -CN A₂A₂ and A₂B.

CSN3 Locus

- *Milk yield:* K-CN AB>BB>AA. Statistically highly significant differences ($p < 0.001$) recorded between K-CN AB and BB, respectively between K-CN AB and AA.
- *Fat content:* BB>AB>AA. The differences between the three genotypes were statistically highly significant ($p < 0.001$).



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- *Protein content.* Cows with K-CN BB genotype produced a milk with higher protein content compared to cows with K-CN AB and AA genotypes. Statistically significant differences ($p < 0.05$) recorded between K-CN BB and AB, respectively AA genotypes.
- *Casein content.* There were no statistically significant differences ($p > 0.05$) between genotypes for this trait.
- *Lactose content* was higher in the milk sampled from heterozygous individuals K-CN AB. Statistically highly significant differences ($p < 0.001$) recorded between K-CN AB and BB genotypes.
- *SCC.* Between K-CN AB and BB genotypes we recorded statistically highly significant differences ($p < 0.001$).

LGB Locus

- *Milk yield:* AA>AB>BB. Statistically highly significant differences ($p < 0.001$) recorded between β -LG AA and BB genotypes and between β -LG AB and BB (942 kg).
- *Fat content:* BB>AA>AB. Statistically distinct significant differences ($p < 0.01$) recorded between β -LG BB and AB.
- *Protein content.* AA>BB>AB. Milk sampled from homozygous individuals for β -LG*A allele had a higher protein content, with approximately 1,9% respectively 1,0% (relative values) compared to milk gathered from cows with β -LG AB and BB genotypes. Statistically significant differences ($p < 0.05$) recorded between β -LG AA and AB genotypes.
- *Casein content.* BB>AB>AA. Statistically significant differences ($p < 0.05$) recorded between β -LG BB and AA genotypes.
- *Lactose content* was higher with 3,7%, respectively with 3,8% (in relative values) in the milk sampled from heterozygous individuals β -LG AB compared to homozygous individuals AA and BB. Statistically distinct significant differences ($p < 0.01$) recorded between β -LG AB and BB, respectively between β -LG AB and AA genotypes.
- *SCC.* Cows with β -LG AA genotype produced a milk with low SCC/mL. Statistically highly significant differences ($p < 0.001$) recorded between β -LG BB and AA, respectively between β -LG BB and AB genotypes.

Romanian black spotted breed (BNR)

CSN1S1 Locus

- *Milk yield.* The homozygous individuals for α_{S1} -CN*B allele produced a higher milk yield compared to heterozygous individuals α_{S1} -CN BC, but there were no statistically significant differences ($p > 0.05$) between the two genotypes for this trait.



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- *Fat content.* Cows with α_{S1} -CN BB genotype produced a milk with 1.5% higher fat content (relative value) compared to cows with α_{S1} -CN BC genotype. The statistically differences were highly significant ($p < 0.001$) between the two genotypes.
- *Protein content.* The milk sampled from heterozygous individuals α_{S1} -CN BC had fat content higher with 1.1% (relative value) compared with milk gathered from cows with α_{S1} -CN BB, the statistically differences between these two genotypes for the mentioned trait being highly significant ($p < 0.001$).
- *Casein content.* Mean values for casein content indicate that the cows with genotype α_{S1} -CN BB produced a milk with 18.8% more fat content (relative value) compared to the milk gathered from cows with BC genotype. Between the two genotypes we recorded statistically highly significant differences ($p < 0.001$).
- *Lactose content* was approximately 1.6% higher (relative value) in the milk sampled from heterozygous individuals α_{S1} -CN BC compared with milk gathered from cow with α_{S1} -CN BB genotype, the statistically differences between the two genotypes for this trait being highly significant ($p < 0.001$).
- *SCC.* Milk produced by cows with α_{S1} -CN BB genotype had a 23,4% (relative value) higher SCC/mL compared with the milk produced by cows with α_{S1} -CN BC genotype. Between the two genotypes the statistically differences were highly significant ($p < 0.001$).

CSN2 Locus

Milk yield: $A_1A_1 > A_1A_2 > A_2A_2 > A_2B > A_1B$. Statistically highly significant differences ($p < 0.001$) were recorded between β -CN A_1A_2 and A_1B respectively A_2B genotypes.

- *Fat content* was with 3,8% (relative value) higher in the milk sampled from cows with A_1B genotype compared to the milk produced by homozygous individuals A_1A_1 ($p < 0.001$). Statistically highly significant differences were recorded as well between A_2B genotype and β -CN $A_1A_1/A_1A_2/A_2A_2$ genotypes.
- *Protein content.* Milk produced by cows with A_1B and A_2B genotypes had a protein content with 3%, respectively 2.7% and 2.3% (relative values) higher compared to milk produced by cows A_1A_1 , respectively A_2A_2 and A_1A_2 genotypes. In all three cases the statistically differences were highly significant ($p < 0.001$).
- *Casein content* was 9.6% higher (relative value) in milk sampled from cows with A_1B genotype compared to milk gathered from homozygous individuals for β -CN* A_1 alleles and with 4.8% (relative value) compared with milk produced by heterozygous individuals A_1A_2 . In both cases the statistically differences were highly significant ($p < 0.001$).



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- *Lactose percent.* We identified positive relationships between A_1A_2 genotype and milk's lactose content. Statistically highly significant differences ($p < 0.001$) recorded between A_1A_2 and A_2A_2 , respectively between A_1A_2 and A_2B genotypes for this trait.
- *SCC.* Milk produced by cows with A_1B genotype had a lower SCC/mL ($p < 0.001$) with approximately 51%, respectively 35% (relative values) compared to milk sampled from heterozygous individuals A_1A_2 , respectively the homozygous ones A_2A_2 . Statistically highly significant differences ($p < 0.001$) recorded between A_2B genotype and A_1A_1 , A_1A_2 , respectively A_2A_2 genotypes.

CSN3 Locus

- *Milk yield:* Homozygous individuals K-CN AA produced the highest milk yield, with about 494 kg more than the heterozygous ones K-CN AB and with 133 kg more than the homozygous individuals for the K-CN*B allele. The statistically differences between these genotypes for the milk yield were highly significant ($p < 0.001$).
- *Fat content:* Milk sampled from homozygous individuals K-CN BB had a higher fat content with 2.7%, respectively 1.3% (relative values) compared to milk produced by cows with the other two genotypes K-CN AA, respectively K-CN AB. Statistically highly significant differences ($p < 0.001$) recorded between BB and AA genotypes, as between BB and AB there were statistically significant differences ($p < 0.05$).
- *Protein content.* K-CN BB>AB>AA. Homozygous individuals with K-CN BB genotype produced a milk with 7.3%, respectively 7% (relative values) higher protein compared to milk produced by cows with AA, respectively AB genotypes. The statistically differences between K-CN BB and AB genotypes were distinct significant ($p < 0.01$).
- *Casein content:* K-CN BB>AB>AA. Statistically highly significant differences ($p < 0.001$) recorded between K-CN BB and AB, respectively between K-CN BB and AA genotypes.
- The *lactose content* was higher in milk produced by homozygous individuals K-CN AA. The statistically differences between K-CN AA and AB were significant ($p < 0,05$) for this trait.
- *SCC* was lower in the milk sampled from cows with AA genotype. Between the three genotypes identified at CSN3 locus the statistically differences were highly significant ($p < 0.001$) for this trait.

LGB Locus

- *Milk yield:* Cows with heterozygous genotype β -LG AB produced with 304 kg more milk compared to cows with β -LG AA genotype and with 214 kg more than the cows with β -LG BB. The statistically differences being highly significant ($p < 0.001$).
- *Fat content:* The homozygous individuals β -LG BB produced a milk with higher fat content compared to the heterozygous β -LG AB and homozygous β -LG AA ones.



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Statistically highly significant differences ($p < 0.001$) were recorded between β -LG BB and AB genotypes.

- *The protein content* was higher in the milk sampled from cows with β -LG BB compared to other two genotypes β -LG AB and AA. Statistically highly significant differences ($p < 0.001$) were recorded between β -LG BB and AA, respectively AB genotypes for this trait.
- *Casein content*: BB>AB>AA. The cows with β -LG*B allele in double dose produced a milk with higher casein content reported to cows that presented the same allele in single dose and the cows with heterozygous genotyped resulted from the combination of the two alleles. The statistically differences between the three genotypes BB>AB>AA were highly significant ($p < 0.001$).
- *Lactose content*. AA>BB>AB. Statistically highly significant differences ($p < 0.001$) were recorded between β -LG AA and AB respectively BB genotypes.
- *SCC*. Milk produced by cows with β -LG AB genotype had the lowest SCC/mL (BB>AA>AB). Between β -LG AB and AA, respectively BB genotypes the statistically differences were highly significant ($p < 0.001$).