ABSTRACT OF Ph.D.THESIS

STUDY OF POLYMORPHISM AND GENETIC STRUCTURE FOR THE LOCUS HEMOGLOBIN AND SERUM TRANSFERRIN FOR THE ROMANIAN SPOTTED BREED

Scientific coordinator: Prof.dr.ing. Gheorghe Mureșan

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STUDY OF POLYMORPHISM AND GENETIC STRUCTURE FOR THE LOCUS HEMOGLOBIN AND SERUM TRANSFERRIN FOR THE ROMANIAN SPOTTED BREED

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The paper with the title “Study of Polymorphism and Genetic Structure for the locus Hemoglobin and Serum Transferrin for the Romanian spotted breed” is structured by three parts, and the first part, with a share of 23.88% concerns aspects dealing with actualities and perspectives in cattle rearing within Romanian conditions and worldwide, and also with importance and heredity of the biochemical systems.

The research was conducted during the 2003 - 2009, on biological material represented by Romanian Spotted breed- Simmental type in the county of Cluj.

Under the goal of characterizing populations of Romanian Spotted breed- Simmental type reared in the Romanian, we took into consideration the results of official control of production for milk production, conducted by the County Offices for Animal Reproduction and Selection.

The dairy cattle rearing are one of the most important branches of livestock breeding, worldwide. This situation became real thanks to development and application of the scientific outputs concerning selection, breeding and improvement of dairy cattle. To enhance the effectiveness of animal rearing methods, the following strategic approaches are developed: development of long-term selection and increase production programmers using the principles and methods based on genetics, testing bulls according to their offspring production performance and qualities, using advanced biotechnologies techniques.
An important role in increasing milk production is represented by the enhanced production based on the use of the world genetic fund to crossings with the aim of improving local breeds with low productive potential.

From the economical point of view, in terms of industrial technology for milk production, maintenance of annual productivity in cows with 4000 kg of milk is not justified.

The business conducted by the operators in milk production will be profitable only when, cattle populations will be enhanced with highly productive individuals, adapted to specific conditions of industrial technology, cattle that would ensure a high content of fat and protein in milk, which would eliminate the heavy and light milk resulted from modern milking facilities and would better convert the administered fodder.

The efficacy of dairy cattle rearing largely depends on genetic potential of the breeds, the value of the genotype, and genetic improvement methods.

The cattle rearing in Romania is an important branch of the agriculture and very important of the livestock sector, by providing very high biological value food products of raw material for food, light and pharmaceutical industries.

According to the bibliographic data, protein polymorphism along with blood groups, represent an objective index in the study of the genetic improvement process in order to create cattle breeds and types. These indices having a constant character during life, is a successful model for studying genetic populations, making genetic crosses, performing surveillance studies in order to obtain information concerning interracial heritage, to create new types of alleles and genotypes.

The polymorphic protein systems are used in carrying out monitoring of the hereditary genetic information, the assessment of distance and genetic similarity, the study of processes at various stages of improvement of the micro-evolution of cattle.

The implementation of the methods that determine the differentiation of the genetic structure of breeds, types, lines, and populations allows to exclude the non programmed interracial crosses, maintain unique alleles responsible for adaptation to local conditions, track distance and genetic similarity of populations, perform animal
grouping in order to correct program improvement in certain phases of the improvement process, based on the genetic and calculated genetic indices.

Taking into consideration the importance of the biochemical polymorphism in fundamental and applied level by using it as a tool in raising the technical level of selection, results obtained so far in this field of research, and especially research conducted subsequent conflicting views on the correlative relationship between biochemical systems and some economic traits and somatic work, aims to establish the biochemical (genetically) profile determined at the hemoglobin and transferrin loci in Romanian Spotted breed- Simmental type population from S.C. Basto S.R.L and establish correlative relationship between studied biochemical systems and morpho-productive traits with the aim of using transferrin and hemoglobin phenotypes and genotypes as genetic markers in the work of cattle improvement programmers.

In order to characterize the main morphological and productive traits of Romanian Spotted breed - Simmental type cattle population, research has actually included a total of 321 cows kept and reared in a private firm, S.C. Basto S.R.L., framed in the elite category.

In this paper we established the main morpho-productive traits in dynamics, by three lactations. The effect of half-sib family structures has been studied in terms of the main features of reproduction activities and those of the quantitative and qualitative production in dynamics, by three lactations, on offspring resulted from four bulls.

Determination of hemoglobin phenotype was done by paper electrophoresis using Medina-Veronal-Tris buffer reagent, at pH = 8.6, f.i. = 0.05. Migration time was 18 hours and the voltage and current intensity of 200V and 20mA.

The type of the hemoglobin was established based on blood samples collected on anticoagulant. Preparation of collected hemoglobin samples was performed by three successive washes with saline solution and centrifugation at 3000 rpm. The resulting sedimented erythrocytes were hemolyzed with distilled water in 1/9 parts, ratio. Staining was done with Amidoschwarz bands 10B and discoloration by passing them through successive baths of aqueous 7% acetic acid.
Genetic variants of serum transferrin were determined using the method of polyacrylamide gel electrophoresis (SDS-PAGE), 8% and 0.8 mm thick.

The Commercial Society Basto S.R.L. was founded in 1992, and its activity profile is cattle rearing. It is located in the terrace of Somes between of Cluj-Napoca town and Apahida village, to the left of the road E-60. The biological material is represented by Romanian Spotted breed - Simmental type, and organized in a flock of 407 heads, of which: 339 cows, 38 heifers, 30 young females of different ages.

During the first lactation, the investigated biological material performance is characterized by good production and reproduction, certified by the mean quantities of milk \((4514.95 \pm 35.31 \text{ kg})\), fat \((178.40 \pm 1.27 \text{ kg})\), protein \((152.15 \pm 1.12 \text{ kg})\) and dry \((573.85 \pm 5.94 \text{ kg})\) products. The first calving was recorded at 938.14 \(\pm 11.80\) days of age, 31-32 months respectively. During lactation, with a duration period of 336.28 \(\pm 12.31\) days, an average daily production of 14.80 \(\pm 0.155\) kg with a maximum daily milk production of 22.21 \(\pm 0.674\) was achieved. By paternal relatedness groups, in overall, best performance were recorded in 51116 bull daughters (T1) and lowest, in daughters of the 48129 bull (T4).

During the second lactation, under natural growth of production parameters, corresponding to age and lactation, both hierarchy of the mean values and variability of studied traits, in overall and by paternal relatedness groups remain unchanged compared to the performances obtained in the first lactation, except lactation duration, which, in this case, is longer by 0.4%.

During the third lactation, the studied biological material exhibits higher values in all quantitative traits analyzed, recording increases values, compared with the first lactation, being situated between values 20.06\% for average daily production of milk and 19.65\% for the amount of fat by normal lactation. By paternal relatedness groups, during this lactation, the best performances were recorded in daughters of the 51119 (T2) bull, and lowest in daughters of the 48129 (T4) bull.

The quantitative milk production, whatever we talk about normal lactation and total lactation, has high amplitude and the average by total lactations reached good levels.
with over 500 kg higher than the level achieved by cows herds included in official control of production (OCP) performed for this breed at county level. In dynamics, the maximum output is achieved during the third lactation reaching the level of 5,937.85 ± 27.88 kg by total lactation, and 5,417.94 ± 22.20 kg by normal lactation.

The mammary rest, by the entire cattle population from S.C. Basto S.R.L., recorded an average of 58.34 ± 0.99 days, meeting the minimum value into the forth lactation, meaning 54.16 ± 2.07 days, and a maximum during the first lactation, of 68.57 ± 3.54 days.

Calving interval recorded an average of 393.15 ± 4.15 days, and the minimum was noticed during the forth lactation, being of 385.14 ± 3.29 days, while the maximum value was notice during the first lactation, 404.85 ± 4.46 days.

The average age when the first calving occurs in the studied Romanian Spotted breed - Simmental type cow population was of 938.14 ± 11.80 days.

Table 18
Dynamics of main indicators of livestock production and breeding of cows
SC BASTO SRL

<table>
<thead>
<tr>
<th>Attribute</th>
<th>UM</th>
<th>Lactation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>1</td>
</tr>
<tr>
<td>N cap/head</td>
<td>125</td>
<td>111</td>
</tr>
<tr>
<td><strong>Total lactation</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DLT zile/days</td>
<td>336.28±12.31</td>
<td>337.65±10.88</td>
</tr>
<tr>
<td>Quantity milk kg</td>
<td>4978.31±63.18</td>
<td>5650.45±28.42</td>
</tr>
<tr>
<td>Fat %</td>
<td>3.94±0.01</td>
<td>3.94±0.01</td>
</tr>
<tr>
<td>Content fat kg</td>
<td>196.14±1.54</td>
<td>222.62±1.18</td>
</tr>
<tr>
<td>Protein %</td>
<td>3.39±0.01</td>
<td>3.32±0.01</td>
</tr>
<tr>
<td>Content protein kg</td>
<td>168.76±1.31</td>
<td>187.59±0.95</td>
</tr>
<tr>
<td><strong>Normal lactation</strong></td>
<td></td>
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<tr>
<td>DLT zile/days</td>
<td>304.98±0.36</td>
<td>304.87±0.30</td>
</tr>
<tr>
<td>Quantity milk kg</td>
<td>4514.95±35.31</td>
<td>5101.89±22.43</td>
</tr>
<tr>
<td>Fat %</td>
<td>3.95±0.01</td>
<td>3.94±0.01</td>
</tr>
<tr>
<td>Content fat kg</td>
<td>178.40±1.27</td>
<td>201.01±0.93</td>
</tr>
<tr>
<td>Protein %</td>
<td>3.37±0.01</td>
<td>3.31±0.01</td>
</tr>
<tr>
<td>Content protein kg</td>
<td>152.15±1.12</td>
<td>168.87±0.31</td>
</tr>
</tbody>
</table>

Age first calving (VPF)=938.14±11.80 zile/days
Analyzing the genetic structure for the hemoglobin and transferrin loci in analyzed biological material, researched based on the recorded phenotypes, the hemoglobin gene frequencies and genotypes were determined. The prevalence of the HbAA homozygous type and HB^A gene was found, the HB^B allele being present in both generations, but with a low frequency. Within the parent population studied, the HB^A gene frequency is 0.913 and the HB^B frequency of 0.087. The frequency of the Hb^A Hb^A genotype and frequency of the gene HB^A generation was found 6.47% higher in offspring generation. This increase of the homozygous trait and HB^A allele frequency are due to the genetic structure of the male parents, this being strongly affected by the artificial component of the selection.

The gene frequency at the transferrin locus is of 0.629 Tf^D; Tf^A 0.365 and 0.006 Tf^E. In offspring population, the gene frequency at the hemoglobin locus is 0.945 Hb^A and 0.055 Hb^B, and at the transferrin locus Tf^D 0.617; 0.377 Tf^A, and 0.006 Tf^E.

Based on the consistency test, the estimated value of $\chi^2$ is lower than the corresponding admitted safety threshold value obtained from table data. This means that the research material is in genetic balance for the hemoglobin and serum transferrin loci. The sample extracted of the population is consistent, in terms of gene and genotypic distribution of hemoglobin and transferrin locus, with the population as a whole, being representative for the Bâlțăță românească cattle - Simmental type population.

### Table 27

<table>
<thead>
<tr>
<th>Bull code</th>
<th>Genotype</th>
<th>Total</th>
<th>$\chi^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Hb^A Hb^B</td>
<td>Hb^B Hb^B</td>
<td>Hb^A Hb^B</td>
</tr>
<tr>
<td>51616 T1</td>
<td>observed</td>
<td>48</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>expected</td>
<td>48,12</td>
<td>4,77</td>
</tr>
<tr>
<td>51119 T2</td>
<td>observed</td>
<td>45</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td>expected</td>
<td>45,16</td>
<td>5,66</td>
</tr>
<tr>
<td>19942 T3</td>
<td>observed</td>
<td>37</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>expected</td>
<td>37,06</td>
<td>2,88</td>
</tr>
<tr>
<td>48129 T4</td>
<td>observed</td>
<td>18</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>expected</td>
<td>18,18</td>
<td>3,64</td>
</tr>
<tr>
<td>TOTAL</td>
<td>observed</td>
<td>148</td>
<td>18</td>
</tr>
<tr>
<td></td>
<td>expected</td>
<td>148,24</td>
<td>17,26</td>
</tr>
</tbody>
</table>

$\chi^2_{tab}=5,99$
Distribution of transferrin geotipurilor consistency and test the survey population

<table>
<thead>
<tr>
<th>Bull code</th>
<th>Genotype</th>
<th>Total</th>
<th>$\chi^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$Tf^{AT}f^A$</td>
<td>$Tf^{AT}f^B$</td>
<td>$Tf^{AT}f^e$</td>
</tr>
<tr>
<td>51616 T₁</td>
<td>observed</td>
<td>10</td>
<td>20</td>
</tr>
<tr>
<td></td>
<td>expected</td>
<td>8,74</td>
<td>18,14</td>
</tr>
<tr>
<td>51119 T₂</td>
<td>observed</td>
<td>8</td>
<td>19</td>
</tr>
<tr>
<td></td>
<td>expected</td>
<td>7,44</td>
<td>18,85</td>
</tr>
<tr>
<td>19942 T₃</td>
<td>observed</td>
<td>7</td>
<td>16</td>
</tr>
<tr>
<td></td>
<td>expected</td>
<td>6,02</td>
<td>14,98</td>
</tr>
<tr>
<td>48129 T₄</td>
<td>observed</td>
<td>2</td>
<td>12</td>
</tr>
<tr>
<td></td>
<td>expected</td>
<td>1,64</td>
<td>11,63</td>
</tr>
<tr>
<td>TOTAL</td>
<td>observed</td>
<td>27</td>
<td>67</td>
</tr>
<tr>
<td></td>
<td>expected</td>
<td>23,59</td>
<td>63,19</td>
</tr>
</tbody>
</table>

In this thesis, we have established the main features of the morpho-productive traits, in dynamics, by three lactations. During the first lactation, according to the hemoglobin phenotype, we observed that the homozygous individuals of the $Hb^A$ allele performed quantitative superior yields compared with the $HbAB$ individuals, by: 336.81 kg physically milk (7.96%), 11.61 kg fat (6.88%) 8.74 kg protein (6.06%) and 43.65 kg dry matter (8.13%) by normal lactation. Also, the average and maximum daily production are greater with 9.97% and 12.68%. From reproductive point of view, essential differences are not noticed, the value of the reproductive indices is I advantage of the individuals $HbBB$ and $HbAB$ being with 0.78% higher for first calving age and -4.71% for the duration of lactation.

According to transferrin phenotype, the best performing production performances were obtained by $Tf^D$ allele homozygous individuals and the lowest, except for protein content, to the individuals in possession of homozygous allele $Tf^A$. The $TfDD$ homozygous individuals performed quantitative normal lactation yields higher by: 345.4 kg physically milk (8.07%) 14.92 kg fat (8.9%) 11.21 kg protein (7.75%), 40.74 kg dry matter (7.48%) and an average maximum daily production of physical milk higher by 3.92% and 6.55%, compared with $Tf^A$ individuals.

Concerning the studied traits, the $TfAD$ heterozygous individuals occupy intermediate position within homozygous groups, except the maximum yield of daily milk...
production, dry matter content and fat content, traits for which, maximum values were recorded, while the protein content had the lowest values.

The analysis of data concerning the simultaneous influence of hemoglobin and transferrin allele reveals a series of aspects. One of them is that the best productive performances are realized by the transferrin groups combined with HbAA phenogroup. The most prominent differences are found between HbAATfAA homozygous individuals and TfAAHbAB heterozygous. These differences, in the advantage of the first group, being the following: 899.83 kg physical milk (20.33%), 32.36 kg fat (18.69%), 28.1 kg protein (18.91%), and 111.14 kg dry matter (19.83%). The TfAAHbAB individuals are characterized by a slight precocity. The reproductive age of first calving was lower by 13.69% and duration of lactation with 15.09% shorter.

During the second lactation some changes in ranking groups could be noticed. Thus, function of hemoglobin phenotype, the highest values was achieved by HbBB and HbAB individuals in all analyzed traits.

According to transferrin phenotype, we found that the Tf^D allele homozygous individuals had the best performing production in this lactation, too. The homozygous of Tf^A allele recorded superior average values, compared to heterozygous individuals for all traits of the dairy production.

The homozygous and heterozygous individuals’ possessors of the Hb^B allele have a 15.23 days shorter lactation period, compared to Hb^A homozygous individuals.

In groups structured according to transferrin type, the differences between Tf^A allele homozygous individuals and TfAD allele heterozygous individuals ranges from 3.21% for normal lactation fat production and 2.47% for milk production by normal physical lactation in advantage of the TfAA homozygous individuals. The average and maximum daily milk production are higher in TfDD phenotype, by 0.6 kg (3.52%) and 1.23 kg (4.85%), respectively in advantage of TfAD phenotype, while duration of lactation was almost equal within all three groups analyzed.

Simultaneous analysis of data concerning the influence of transferrin and hemoglobin phenotype shows that in this lactation, within the transferrin groups from the
combination with HbA phenotype the hierarchy recorded during the first lactation was noticed in this lactation, too. Even though, a reduction of differences was recorded, and the transferrin groups combined with HbBB and HbAB phenotypes, had a changed hierarchy and recorded higher values in individuals with and HbAB and HbBB with TfAA and lowest in HbAB and HbBB individuals with TfAD.

By a normal duration, during the second lactation, the HbAATfDD individuals had in average a higher production than HbAATfAA individuals, by: 122.45 kg milk physical (2.45%), 1.3 kg fat (0.65%), 4.53 kg protein, (2.76%) 8.33 kg dry matter (1.32%). They have a 10.3 days greater duration of lactation (3.21%) and achieved a superior average and maximum daily production of physical milk, by 0.54 kg (3.33%) and 1.32 kg (5.68%), respectively.

Within the transferrin combinations with HbAB and HbBB phenotypes, the group of the TfAA homozygous had higher production compared with the TfAD heterozygous group, by: 989.26 kg physical milk (20.31%), 36.39 kg fat (18.54%), 25.14 kg protein(15.05%), 122.02 kg dry matter (19.74%). They are characterized by a longer lactation period of 93.09 days (27.73%), and during this period they realized an average and maximum daily physical milk production with 2.3 kg (13.99%) and 2.6 kg (10.69%), respectively.

It was found that individuals of all ages have HbAA transferrin phenotype combined with production below the corresponding group and HbBB HbAB combination. In individuals with TfAAHbAB + BB phenotype, concerning milk production quantitative traits, differences between 15.79% were recorded for maximum daily production, and 33.78% for the duration of lactation, these oscillating between 16.70% and 19.61%. In individuals with TfDDHbAB + BB phenotype, the differences range from 8.85 % for lactation duration and 8.83% for average daily production. The more reduced differences were recorded between TfAD groups within the two hemoglobin phenotypes, these having values between 0.06% in average daily production of physical milk and 1.07% in protein content.
During the third lactation, depending upon the hemoglobin phenotype, the highest performances were recorded by the HbAB individuals, differences in production compared to HbAA individuals, in what concerns the standard deviation, being: 329.95 kg physical milk (6.15%), 17.52 (8.31%) kg fat, 14.23 kg protein (8.08%), and 46.94 kg dry matter (6.96%). In terms of reproductive capacity, in conditions of the same calving interval, the HbAB individuals have higher duration of lactation, by 0.91% (3.04 days).

According to transferrin phenotype, the group of the Tf^D allele homozygous, by normal lactation, performed yields higher average productions compared to Tf^A allele homozygous, by: 814.31 kg physical milk (16.76%), 104.79 kg dry matter (17.13%), 28.77 kg fat (14.73%), 29.46 kg protein (18.55%). The average and maximum daily production, for individuals with TfDD homozygous phenotype is greater with 41.36% (6.01 kg) and 25.33% (5.46 kg), respectively compared to TfAA homozygous individuals. Within conditions of the same calving interval, in both compared groups, the TfDD homozygous individuals recorded a shorter average duration of lactation, by 0.24% (0.83 days).

Analyzing the simultaneous influence of hemoglobin and transferrin phenotypes on production traits, we can notice, in this lactation too, the superior performances of the transferrin combinations with HbAB phenotype. In both groups Tf^D allele homozygous individuals can be noticed due to their achieved performances. Thus, by the normal duration of this lactation, the HbAATfDD individuals produced higher average yields compared to the with HbAATfAA individuals: 942.72 kg physical milk (20.50%), 33.37 kg fat (18.10%), 31.38 kg protein (20.87%), 116.3 kg dry matter (20.03%). The individuals HbAATfDD realized a daily average and maximum milk production higher than HbAATfAA individuals, by 51.27% (7.06 kg), and 26.06% (5.38 kg), respectively.

Within HbAB hemoglobin phenotype, the Tf^D allele homozygous individuals have higher production compared to TfAD heterozygous individuals, by: 1264.54 kg milk physical (25.31%), 179.73 kg dry matter (28.71%), 53.46 kg fat (26.88%), and 58.88 kg protein (36.60%). The TfDD homozygous individuals belonging to the hemoglobin HbAB phenotypes achieved higher quality productions compared to HbAA phenotype.
The body conformation traits have the same average values in both groups of individuals formed on the basis of hemoglobin phenotype, Hb^A allele homozygous individuals and Hb^B allele heterozygous individuals, respectively. Except trunk length, thorax perimeter, width and length of leg, the rest of conformation traits are significantly higher in Hb^B allele owners.

According to transferrin phenotype, the average values of the conformation traits are very similar. The differences among them were between 0.96% for height at withers and 3.62% for body weight. By groups, except body weight, croup length, and thorax perimeter, the Tf^D allele homozygous individuals have the highest dimensional values compared with Tf^A allele homozygous individuals, which is characterized by a lower somatic development. The TfAD heterozygous individuals, recorded intermediary values between both groups, except waist, hip grinding width, thorax perimeter and body mass.

Whatever hemoglobin phenogroup, the groups of the TfDD and TfAD, which include specific individuals, is characterized by a better body development compared to TfAA homozygous phenotype owners. The lowest values of the studied conformation traits are found in HbAB + BBTfAA individuals.

The udder conformation, analyzed in terms of development and morphological symmetry, is similar in groups of individuals with different hemoglobin phenotype. A similar situation is encountered in the groups constituted according to transferrin phenotype, too. The Tf^D allele homozygous individuals are characterized by a more developed and extended udder with front and rear spaced nipples, and a corresponding perimeter. Compared with them, the parameters of the TfAA homozygous individuals are characterized by lower udder, heterozygous group presenting intermediate values.

The knowledge of the phenotypic correlations between characters is particularly important for animal breeding methodology and technique. They are being based on the study of the causal, genetic and environmental factors, which determine the interdependence between the characters. In the mean time, the phenotypic correlations provide information concerning the meaning and degree of correlation between characters.
The analysis of the influence of the hemoglobin phenogroup during the first lactation on phenotypic correlations between investigative traits reveals the followings: the way of traits grouping, their sequence and way of association of the traits groups that are correlated one with each other. All those approached and described issues present a series of features by the two categories of considered individuals.

The intense positive correlations recorded in both groups were found between: quantitative traits of milk production, thorax width, the index of the transversal trunk shape (IFTT) and basin-thorax index (IBT), thorax depth and index of thorax depth (IAT), anterior and posterior nipple length, duration of lactation and calving interval.

Positive and average correlations are recorded between qualitative characteristics of milk production, between average daily maximum physical milk production and udder length; between waist and oblique trunk length; body weight and greatness index (MI); between age of first calving (VPF); between perimeter of the thorax and index of the thorax depth (IAT); between the index of the basin-thorax (IBT) and bone index (OI); and between compactness index (CI) and thorax depth index (IPT). The other traits or groups of traits are not correlated; either have very low intensity correlations. In HbAA individuals, the quantitative characteristics of milk production traits are correlated with the group of traits that exhibit the development and proportionality of the thorax.

During the first and third lactations (I–III), the phenotypic correlations between production traits, breeding and conformation, by total population and by HbAA individuals except the index of the body lateral conformation (IFCL) are positive. The most intense correlations were recorded between quantitative and qualitative traits of milk production, and between them and duration of lactation, their levels being between 0.998 and 0.781. Intense positive correlation were also recorded between thorax width, the index of the transversal trunk conformation (IFTT) and basin – thorax index, between the anterior and posterior nipple distance, between anterior and posterior nipple length, between thorax depth and index of thorax depth (IAT).

Medium positive correlations are found between absolute and relative values of thorax depth and age of first calving (VPF), body mass and greatness index (MI) between
waist and oblique trunk length, and between the absolute and relative value of the thorax width and bone index (IO). Among other recorded traits or groups of traits, correlations are lacking or are of very low intensity.

During the first lactation, the influence of the transferrin phenotype on interrelations between the studied traits causes marked differences between how the correlated traits are combined by groups and succession of traits groups by categories of studied individuals, without major changes in direction and intensity of correlations.

In TfDD individuals, positive and intense correlations were recorded between quantitative and qualitative traits of milk production and duration of lactation, with values between 0.996 and 0.796, and between chest width, IFTT and IBT. Moderate correlations were found in: milk protein content and milk fat and dry matter content, quantitative traits of milk production with VPF, thorax depth and thorax perimeter.

In TfAD individuals positive and intense correlations were noticed between: quantitative and qualitative traits of milk production and lactation length, with values between 0.999 and 0.802; between anterior and posterior nipple length, between thorax depth and IAT, and between thorax width, IFTT and IBT.

The degree of determination quantifies the extent in which a variable, considered as independent, explains the variations of a variable allele, considered as dependent. The degree of multiple determinations expresses the proportion of variation of the dependent variable, which can be explained by changes in independent variables while the coefficient of partial determination quantifies the net contribution, of an independent variable in explaining the dependent variable provided other variables are maintained constant.

The coefficient of multiple correlation estimates the simultaneous influence of independent variables on the dependent variable.

The influence of the hemoglobin and transferrin alleles upon the degree of determination and multiple correlations for the traits of body conformation and udder was estimated for these traits both by themselves and the interdependence of production and reproduction traits.
The analysis of data concerning independent and simultaneous influence of hemoglobin and transferrin phenotypes on the degree of determination and multiple correlation coefficients for height at withers and body size, show differences in most cases. They are very significant and significant by considered gene phenotypes, both in terms of multiple and partial determination as well as their multiple correlation intensity.

By total population, the analyzed body size demonstrates a partial determination of the height at the withers that ranges from 22.3% for diagonal length of the trunk and 1.3% for body mass.

In HbAB + BB TfAA, TfDD, and HbAATfDD individuals, height at withers is the most strongly determined by the oblique length of the trunk (between 17.7% in HbAATfDD individuals and 47.1% in TfAA individuals), in HbAA, TfAD and HbAB + BBTfAD individuals, it is most strongly determined by the depth of thorax (between 24.2% in HbAA individuals and 46.8% in HbAB + BBTfAD individuals) and in HbAATfAA individuals is determined by the perimeter of the thorax (39.0%).

Regarding the degree of multiple determinations against the entire population (37.5%), the height at withers is determined by body size analyzed in the highest degree in HbAB + BBTfAD (79.4%) and HbAATfAA (59.7%) individuals and in the lowest degree in HbAATfDD (27.3%) and TfDD (24.6%) individuals.

According to transferrin phenotype, while in TfDD and TfAA individuals, the height at withers is strongly and medium correlated in both cases with oblique length of the trunk and thorax perimeter, in TfAD individuals it is strongly correlated with all analyzed dimensions, from which the most intense relations are with thorax depth.

During the first lactation, the body weight, by total population is determined by the assembly of the investigated traits in an extent of 47.5%, the quotas individual framing between 12.7% for greatness index (MI) and 1.1% for first calving age (VPF). By the groups considered function of hemoglobin and transferrin phenotype, the degree of multiple determination is between 45.9% in HbAA individuals and 87.3% in HbAATfDD individuals, the maximum values for determining individual rates are between 13.4% for
greatness index (MI) in HbAA individuals and 29.2% for thorax perimeter in TfDD individuals.

During first and third lactations (I–III), except HbAATfAD and TfAD individuals, the independent and simultaneous influences of hemoglobin and transferrin allele on the degree of determination recorded lower values, the index of the body weight being mainly determined by the morphological indices, udder indices and body indices. Except HbAATfDD and TfDD individuals, the coefficients of multiple correlations during first and third lactations (I – III) recorded higher values compared to those recorded during the first lactation. The production and reproduction traits do not compete to determine body mass, also except TfAD and HbAATfAD individuals.

The production physical milk by normal lactation, during first lactation, regardless hemoglobin and transferrin groups, or their combination, in all considered groups it is determined by the production of dry matter, except TfAD individuals, in which together with the dry matter production by normal lactation, a low influence (1,2%) also was recorded for the milk dry matter content. During first and third lactations (I – III) the physical milk production is determined by the quantity of dry matter by normal lactation in proportion of in 99.4% -99.9% in all considered groups.

The fat amount during the first lactation is determined by the production of dry matter by normal lactation, with rates ranging between 1.2% and 5.1% associated to milk fat content. The highest values of the degree of determination and coefficient of multiple correlations are recorded in HbAATfAD and TfAD individuals, and the lowest in HbAATfDD and TfDD individuals. During the first and third lactations (I – III) the amount of fat by normal lactation is exclusively and at higher rates (98.6% - 98.9%) determined and correlated with the quantity of physical milk by normal lactation.

The amount of protein during the first lactation, by normal lactation is largely determined (between 91.3% and 97.8%) by the dry matter production by the normal lactation, with rates ranging between 1.3% and 8.4%, and it is associated to the milk protein content. In TfAD group of individuals, the analyzed trait is determined by the amount of physical milk by normal lactation. By the interval included between first and
third lactations (I–III) production of dry matter by normal lactation is exclusively determined and correlated at superior rates with the amount of protein by normal lactation.

*The amount of dry matter* taken into consideration by normal lactation in both first lactation and during first and third lactations (I–III) is determined and correlated with physical milk production by normal lactation.

*The milk fat content* by first lactation, by all individuals, is determined by a group of complex factors, the investigated characteristics determining its traits in 60.3% share, where the largest influence is played by: dry matter milk content (18%), physical milk production (8.4%), length of anterior nipples (3.3%), fat production by normal lactation (3.2%), duration of lactation (1.7%), udder length (1.7%), croup width at the hips level (1.1%) and thoracic perimeter (1%). By total population and in HbAA individuals, the milk fat content is highly correlated with the dry matter milk content. In HbAATfDD and TfDD individuals it is the most intense correlated with physical milk production by normal lactation, and in HbAATfAD and TfAD individuals, with the distance between the posterior nipples. During the first and third lactations (I–III), the fat milk content is almost entirely determined by the dry matter milk content.

*The milk protein content* during the first lactation is determined by the investigated traits in an extent of 83.5%, of which, the interdependence relationship are exhibited by: the fat production by normal lactation, thorax depth, distance between posterior nipples, average daily physical milk production, physical milk production by normal lactation, dry matter production by normal lactation and the dry matter content of milk. The degree of determination of the characteristics of all analyzed groups of individuals varies considerably between 38.2% and 86.1%. The lowest values were recorded in HbAA and TfDD individuals and similar values and/or higher values in TfAD, HbAATfDD, and HbAATfAD individuals and by total population, too. During the first and third lactations (I–III) the milk protein content is almost entirely determined and in all categories of considered individuals, by total dry matter milk content.
**The dry matter milk content** during the first lactation, by total individuals, is determined, in 72.0% share, by the investigated traits, of which, decisive action is played by: dry matter production by normal lactation, milk fat content, the udder high perimeter (PMU), height at withers (IG), the average calving interval (CI), udder length (Ln.U.), the physical milk production by normal lactation and croup width at hips level (Lr.C.ş.). By considered the groups, the total degree of determination varies between 52.3% and 84.9%, the minimum values being recorded in HbAA and TfAD individuals, intermediate by total population and in individuals HbAATfAD, and maximum in TfDD and HbAATfDD individuals. During the first and third lactations (I–III), the dry matter milk content is almost entirely determined and in all categories of individuals that were taken into consideration, by milk fat content.

The equations of the linear regression between major quantitative indices of the milk production and lactation traits investigated, within the first lactation, signify the presence of positive stable relationships that are very significant, by categories of considered individuals, common or specific to the analyzed indices, as follows:

- average daily physical milk production is directly determined, very significant and positive by the quantitative physical milk production by normal lactation and negative during lactation;

- the maximum daily milk production and investigated characteristics show positive, significant interdependence relationships with physical milk production by normal lactation and negative with anterior nipples length;

- the milk fat content shows significant and positive relationship with the fat production by normal lactation, milk dry matter content by normal lactation and negative with quantity of physical milk by normal lactation;

- the physical milk production by entirely normal lactation is exclusively and very significant determined by the production of dry matter by normal lactation;

- the fat production by normal lactation is very significant dependent of the dry matter production by normal lactation and of milk fat content;
- the protein production by normal lactation is very significant dependent on production of dry matter by normal lactation and of milk protein content;
- the milk protein content reveals significant and positive relationship with the protein production by normal lactation and milk content in dry matter, and negative with physical milk quantity by normal lactation and dry normal production by normal lactation;
- the production of dry matter by normal lactation is very significant determined by the physical milk production;
- the dry matter milk content and investigated characteristics reveal similar aspects to those mentioned for fat and protein content of milk.

Exceptions were recorded in TfAD individuals, where the amount of protein by normal lactation and the quantity of dry matter by normal lactation are in interdependence relationships with the physical milk production by normal lactation in the first case, and in the second case, with this and the milk content in dry matter, and HbAATfDD individuals, where the amount of fat by normal lactation is very significant determined only by the production of dry matter.

During the first and the third lactations (I–III) a reduction in the rate of physical milk production by normal lactation influence on normal lactation fat production. Differences in terms of share of influence function of the type of hemoglobin and transferrin is found only in TfAD individuals for protein production by normal lactation.

The allelic variability in the transferrin locus and blood hemoglobin locus, this will confer practical applications to polymorphism: the analysis of genetic structure of the populations and individuals, phylogenetic studies and zoological systematic studies; determination of gemelar paternity and homozygous degree; early identification of disease or pathological condition; breeds evolution in time; the dynamics of genetic structure of the population; analysis of the degree of adaptation of some population to the environmental conditions in selection and breeding.