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PHENOTYPIC EVALUATION OF MILK FAT AND PROTEIN TRAITS IN RELATION TO MATERNAL ANCESTRY IN TWO ROMANIAN CATTLE BREEDS

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Abstract: To assess the phenotypic performance of dairy cattle ancestry, data were analyzed for three direct maternal lines: mother (M.), paternal grandmother (PG.), and maternal grandmother (MG.), as fat and protein (percentage and kg) and combined fat and protein yield (kg). The study involved two Romanian breeds, from two different farms, *Bălțată cu negru românească* and *Bălțată românească*; for the first one, the fat and protein contents were as follows: 3.33 ± 0.05 % fat and 3.28 ± 0.02 % protein for M., 4.08 ± 0.11 % fat and 0.00 % protein for PM., and 3.16 ± 0.14% fat and 3.23 ± 0.05% protein for MG. In *Bălțată românească*'s case, the values were: 3.99 ± 0.07 % fat and 3.46 ± 0.07 % protein for M., 4.14 ± 0.06 % fat and 3.55 ± 0.07 % protein for PG., and 3.97 ± 0.17 % fat and 3.32 ± 0.05 % protein for MG. On average, *BR*, the mothers produced 208.9 kg of fat and 167.1 kg of protein per lactation, while *BNR* cows produced 199.4 kg of fat and 160.2 kg of protein. At the grandparental level, maternal grandmothers of *BR* cows produced on average 207.2 kg fat and 165.9 kg protein, while paternal grandmothers produced 210.3 kg fat and 168.4 kg protein; in the *BNR* population, maternal grandmothers produced 198.1 kg fat and 159.6 kg protein, while paternal grandmothers produced 200.8 kg fat and 161.3 kg protein. The ancestry in the farm that raises *BR*, originated from imported Fleckvieh genetics, contributing to superior performance.

Keywords: *Bălțată cu negru românească, Bălțată românească, dairy cattle, fat, milk, protein, quality*

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INTRODUCTION

Cattle milk is rich in nutrients, containing about 100 essential substances for the human body, including 10 fatty acids, 20 amino acids, over 45 minerals, and approximately 25 vitamins [1]. Because of this complex biochemical profile, milk has long been considered one of the most complete foods, contributing not only to human nutrition but also to the development of the dairy industry worldwide. Its balanced combination of macronutrients and micronutrients makes it an essential product in the daily diet of children, adults, and elderly people, ensuring adequate intake of proteins, calcium, and vitamins that are indispensable for growth, bone health, and metabolic balance. Despite the fact that its chemical composition depends on some factors, on average this mammary gland product contains 87.5 % water and 12.5 % dry matter when cow's milk is analyzed (3.3 - 4.5 % fat, 3.2 - 3.4 % protein, 8.4 % lactose, and 1.0 % ash); it has a density that ranges between 1.026 - 1.034 $g \cdot cm^{-3}$ (at 20 ºC), it boils at 100.2 ºC, and freezes at -0.55 ºC [2]. The variability of these values is influenced by breed, stage of lactation, age of the animal, nutrition, and environmental conditions. Thus, both genetic and environmental factors determine the productive potential and nutritional value of bovine milk [1].

Regarding *Bălțată cu negru românească* and *Bălțată românească*, the two cattle breeds that were included in this study, they are considered important in dairy raising domain in Romania, since their milk yields, fat, and protein percentage are satisfying. Literature indicates that milk yield in *Bălțată cu negru românească* ranges between 5,500 - 8,000 liters in 305 days of lactation, although there are individuals that exceed even 10,000 liters per normal lactation [2, 3]. Such productivity levels place this breed in a competitive position when compared with other European dual-purpose breeds. Studies on its milk quality have shown that it typically has 3.5 - 3.9 % and approximately 198 - 312 kg of fat, 3.1 - 3.4 % and approximately 171 - 272 kg of protein [2], which indicates a composition suitable for processing into dairy products such as cheese, yogurt, and butter. Similarly, *Bălțată românească* breed has satisfying meat and milk production yields, and even if it is a mixed breed, the milk quantity per normal lactation ranges between 4,000 - 5,500 liters (and even more than 9,500 liters) [3]. With regard to the milk quality of this breed, values are reported as 3.6 - 3.9 % and approximately 198 - 312 kg of fat, 3.1 - 3.4 % and 171 - 272 kg of protein [1]. These results underline the dual-purpose character of the breed, which combines good milk productivity with a strong aptitude for beef production. For farmers in Romania, this flexibility represents an economic advantage, as it allows adaptation to market demands for either dairy or meat products. Such values are consistent with the average milk composition of many dairy breeds, indicating that these two Romanian breeds are valuable and that they have a balanced nutritional profile. At the same time, they highlight the importance of maintaining and improving these local genetic resources, especially in the context of European Union policies that promote biodiversity, sustainability, and the conservation of native livestock breeds.

The scientific interest in studying milk production goes beyond simple yield measurements. The quality of milk, especially its fat and protein content, is a central parameter for the dairy industry, as it directly affects processing efficiency and the nutritional value of final products. In addition, these traits are moderately heritable, which means that selection programs can significantly improve them over successive generations. The role of maternal ancestry in shaping production traits has received

growing attention in recent years. By analyzing the influence of mothers, maternal grandmothers, and paternal grandmothers on the performance of descendants, researchers can detect valuable patterns that explain variability in milk quality. Maternal effects can be expressed not only through direct genetic transmission but also through epigenetic mechanisms, mitochondrial inheritance, and early developmental influences. These aspects make the study of maternal lineages a valuable tool for animal breeding and for designing strategies that enhance productive efficiency [4].

For Romanian breeds such as *Bălțată cu negru românească* and *Bălțată românească*, investigating maternal ancestry in relation to milk traits has an applied significance, as it allows the identification of superior lineages that can be used as a basis for selection. Improving fat and protein content through genetic strategies ensures higher quality milk for consumers and increased profitability for farmers. Furthermore, such research contributes to the long-term sustainability of the national cattle sector by balancing productivity, product quality, and preservation of genetic heritage.

The aim of this study was to assess the phenotypic expression of milk quality (fat and protein) in relation to maternal ancestry (mother, maternal grandmother, and paternal grandmother) in two Romanian dairy cattle breeds, with the goal of identifying genetic patterns and variation that support selection for improved milk quality. By addressing this objective, the research provides not only scientific knowledge about the breeds studied but also practical recommendations for breeding programs aimed at enhancing milk quality and maintaining the competitiveness of Romanian cattle farming in the broader European context.

MATERIALS AND METHODS

In order to achieve what we aimed for, two cattle breeds were analyzed (29 mothers, 15 paternal grandmothers, and 14 maternal grandmothers from *Bălțată cu negru românească* - farm 1 - and 11 mothers, from *Bălțată românească* - farm 2), from two different farms. Thus, fat and protein percentage and yield (kg) per normal lactation (305 days) were studied, in relation with the ascendence to establish which are the genetic influences and variation that influence the quality of milk.

The fat and protein contents were determined using the MRC Laboratory Automatic Milk Analyzer. The collected data were compiled, statistically analyzed, and interpreted using specific statistical methods; by employing the 2003 Statistical Analysis of Variance and Covariance program, we calculated the arithmetic mean ($\bar{X}$), standard error of mean ($\pm s_x$), standard deviation (s), and coefficient of variation ($V\%$). Also, minimum and maximum values were included in results and discussions to underline the differences between the two cattle breeds and their ascendence.

RESULTS AND DISCUSSION

Various factors influence the fat content in cow's milk. Breed plays a central role, as different cattle breeds are genetically predisposed to produce milk with different fat concentrations; the stage of lactation also plays a significant part, which means that fat levels can fluctuate throughout lactation and are typically higher during the early stages.

Nutritional factors are equally important - cattle that receive a well-balanced, energy-rich diet tend to yield milk with higher fat content. Genetic factors further influence milk composition, including fat percentage, with selective breeding strategies being utilized to enhance specific desirable traits. The overall health status of the cow, particularly in terms of digestive and metabolic function, can impact fat synthesis in the mammary gland; illness or stress conditions may negatively affect both the quantity and quality of milk components. The milking system - including milking frequency, technique, and hygiene level - can also affect milk and fat content, as can seasonal variation in feeding, due to changes in the availability and quality of forage. Furthermore, post-milking handling procedures (as storage, temperature, agitation) may influence milk composition in analysis that is performed. Lastly, both the age of the cow and its lactation rank (as the number of calvings) are relevant; mature cows and those in later lactations tend to produce milk with slightly fat content compared to first-lactation heifers) [5].

The protein content is also influenced by numerous factors: breed plays the most important role, as different cattle breeds exhibit variability in milk protein levels; the stage of lactation is also important, with protein content generally higher during the early lactation period. On the other hand, diet is crucial, as cows which receive a balanced and nutrient-rich diet (particularly one providing adequate protein) are more likely to produce milk with enhanced protein concentration, as dietary protein is essential for milk protein synthesis. Genetic factors contribute notably to milk composition, which can be explained by the fact that the cattle's genetic profile is affecting protein content (selective breeding programs targeting specific traits can consequently influence milk nutritional quality. The health status of the individual, including udder health, also impacts milk composition, as illness or stress can reduce both the quality and quantity of milk constituents, including protein. Also, the milking system and associated hygiene practices are essential to maintain milk quality. Additionally, seasonal variations, such as fluctuations in temperature and humidity, may indirectly influence milk protein levels by affecting the nutritional quality of the cow's diet. Furthermore, post-milking handling and processing methods play a critical role in preserving milk composition, with proper storage and processing necessary to maintain its quality. Understanding and effectively managing these factors are vital for optimizing milk protein content; consequently, procedures often implement targeted management strategies to control these variables, thereby ensuring the production of milk with desirable nutritional characteristics [6].

Table 1 presents data regarding the mean values and variability of milk production indicators of the ancestry for farm 1. It can be observed that the average milk yield for the mother was 6,537.03 ± 222.85 kg, while for the paternal grandmother it registered higher values of 10,370.07 ± 391.75 kg, and for the maternal grandmother, 6,511.36 ± 387.24 kg. Considering the production performances reported in the literature for the *Bălțată cu negru românească* breed - a standard lactation milk yield of 5,000 - 5,700 kg, milk fat content of 3.8 - 3.9 %, and protein content of 3.22 % [6] - it can be concluded that farm 1 possesses valuable ancestry.

Table 1. *Milk production statistics of the ancestry: mothers (M), paternal grandmothers (MT), and maternal grandmothers (MM) for Bălțată cu negru românească cows*

Ascendance	Characters	n	$\overline{X}$	$\pm s_{\bar{x}}$	s	V [%]	Min.	Max.
M.	**Milk [kg]**	29	6,537.03	222.847	1,200.070	18.358	3,776.00	9,234.00
	Fat [%]	29	3.33	0.055	0.294	8.824	2.61	3.99
	Fat [kg]	29	217.31	8.599	46.309	21.310	140.00	336.00
	Protein [%]	29	3.28	0.025	0.134	4.100	2.99	3.54
	Protein [kg]	29	214.00	7.340	39.529	18.472	126.00	303.00
	Fat + Protein [kg]	29	431.31	15.474	83.330	19.320	266.00	639.00
PG.	**Milk [kg]**	15	10,370.07	391.752	1,517.250	14.631	7,757.00	11,551.00
	Fat [%]	15	4.08	0.113	0.436	10.697	3.49	4.40
	Fat [kg]	15	428.20	26.138	101.231	23.641	306.00	508.00
	Protein [%]	0	0.00	0.000	0.000	0.000	0.00	0.00
	Protein [kg]	0	0.00	0.000	0.000	0.000	0.00	0.00
	Fat + Protein [kg]	15	428.20	26.138	101.231	23.641	306.00	508.00
MG.	**Milk [kg]**	14	6,511.36	387.241	1,448.923	22.252	2,915.00	8,595.00
	Fat [%]	14	3.16	0.142	0.533	16.859	2.40	4.12
	Fat [kg]	14	207.64	17.660	66.076	31.822	94.00	309.00
	Protein [%]	14	3.23	0.048	0.179	5.546	3.03	3.78
	Protein [kg]	14	209.57	12.023	44.987	21.466	97.00	273.00
	Fat + Protein [kg]	14	417.21	27.974	104.670	25.088	191.00	582.00

n: number of individuals; $\bar{X}$: arithmetic mean, $\pm s_{\bar{x}}$: standard error of mean, s: standard deviation, *V:* coefficient of variation; min.: minimum value; max.: maximum value.

Table 2 presents the average values and variability of milk production indicators for the ancestry of farm 2. The average production per standard lactation is 7,214.09 ± 298.27 kg for M., 11,113.00 ± 393.16 kg for PG., and 7,464.88 ± 172.20 kg for MG. Analysis of dispersion shows a maximum standard deviation of 1,303.97 kg for PG., 989.24 kg for mothers, and 487.05 kg for MG. The coefficients of variation are 13.71 % for maternal grandmothers, 11.73 % for PG., and 6.53 % for M.

These significant variability values indicate the presence of substantial genetic diversity within the ancestry, which can be exploited to improve productive performance. The relatively high coefficients of variation for PM. and MG. reflect important genetic differences, highlighting clear opportunities for selection based on production criteria. This variability provides a favorable framework for implementing effective genetic improvement programs aimed at increasing milk yield in the descendants.

To maximize results, the use of modern genetic selection methods is recommended, such as pedigree-assisted genetic evaluation and molecular marker analysis (genotyping), which allow the identification of individuals with superior potential even before phenotypic expression. Genomic selection techniques offer increased accuracy in estimating genetic value and can accelerate the improvement process. Furthermore, continuous monitoring of lactation performance, maintaining animal health, and optimal

management conditions are essential for achieving sustainable results. Thus, farm 2 has a solid genetic base that justifies and supports the implementation of rigorous and modern selection strategies to improve milk production and economic performance.

Table 2. *Milk production statistics of the ancestry: mothers (M.), paternal grandmothers (PG.), and maternal grandmothers (MG.) for Bălțată românească cows*

Ascendance	Characters	n	$\overline{X}$	$\pm s_{\bar{x}}$	s	V [%]	Min.	Max.
M.	**Milk [kg]**	11	7,214.09	298.266	989.237	13.713	6,154.00	9,705.00
	Fat [%]	11	3.99	0.069	0.227	5.690	3.51	4.31
	Fat [kg]	11	287.64	11.956	39.654	13.786	248.00	387.00
	Protein [%]	11	3.46	0.073	0.244	7.047	3.13	3.92
	Protein [kg]	11	248.45	8.145	27.012	10.872	208.00	313.00
	Fat + Protein [kg]	11	535.91	19.797	65.660	12.252	456.00	700.00
PG.	**Milk [kg]**	11	11,113.00	393.163	1,303.974	11.734	9,362.00	13,391.00
	Fat [%]	11	4.14	0.056	0.184	4.450	3.92	4.35
	Fat [kg]	11	459.09	15.747	52.227	11.376	377.00	527.00
	Protein [%]	11	3.55	0.070	0.231	6.508	3.26	3.95
	Protein [kg]	11	395.00	16.398	54.386	13.769	339.00	473.00
	Fat + Protein [kg]	11	854.09	31.477	104.397	12.223	718.00	993.00
MG.	**Milk [kg]**	8	7,464.88	172.197	487.046	6.525	6,929.00	8,139.00
	Fat [%]	8	3.87	0.116	0.327	8.453	3.48	4.48
	Fat [kg]	8	289.50	12.327	34.867	12.044	241.00	350.00
	Protein [%]	8	3.32	0.052	0.147	4.418	3.13	3.48
	Protein [kg]	8	247.50	4.444	12.570	5.079	229.00	269.00
	Fat + Protein [kg]	8	537.00	16.548	46.804	8.716	480.00	619.00

n: number of individuals; $\bar{X}$: arithmetic mean, $\pm s_{\bar{x}}$: standard error of mean, s: standard deviation, *V:* coefficient of variation; min.: minimum value; max.: maximum value.

A comparative analysis of milk production data regarding *Bălțată cu negru românească* (farm 1) and *Bălțată românească* breed (farm 2) reveals significant differences in both performance and genetic variability within the ancestry of the *Bălțată cu negru românească breed*. Across all three ancestry categories - mother, paternal grandmother, and maternal grandmother - farm 2 demonstrates superior average milk yields. Specifically, mothers on farm 2 recorded an average yield of 7,214.09 kg, compared to 6,537.03 kg on farm 1; paternal grandmothers yielded 11,113.00 kg on farm 2, surpassing the 10,370.07 kg observed on farm 1; and maternal grandmothers averaged 7,464.88 kg on Farm 6, in contrast to 6,511.36 kg on farm 1. These differences reflect increases of 677 kg, 743 kg, and 953 kg, respectively, indicating a consistently higher production potential in farm 2.

In terms of genetic variability, farm 2 exhibits greater dispersion, as shown by the standard deviations and coefficients of variation. For instance, the highest standard deviation is recorded in paternal grandmothers (1,303.97 kg), followed by mothers

(989.24 kg), and maternal grandmothers (487.05 kg). The coefficient of variation reaches a maximum of 13.71 % in maternal grandmothers, suggesting a broad genetic range that may be exploited through targeted selection. This higher variability reflects a more diverse genetic base and offers increased flexibility for the implementation of improvement strategies.

When comparing these results with the breed standards for *Bălțată cu negru românească* - standard lactation yields of 5,000 - 5,700 kg, milk fat content of 3.8 - 3.9 %, and protein content of 3.22 % [4] - it becomes obvious that both farms exceed the breed average values. However, farm 2 not only surpasses these benchmarks more consistently but does so across all ancestral lines, suggesting the presence of highly productive genotypes. This may be due, at least in part, to the use of Fleckvieh genetics or superior breeding management [5].

Given its higher milk yields and broader variability, farm 2 is better positioned to benefit from modern selection strategies such as pedigree-based evaluation and genomic selection. These methods, including genotyping and the use of molecular markers, allow for the early identification of animals with superior genetic potential, thus accelerating genetic progress. Moreover, continuous monitoring of lactation performance, combined with optimal animal health and management conditions, are essential for sustaining productivity gains.

In conclusion, farm 2 demonstrates both a higher productive performance and a more favorable genetic structure compared to farm 1. These characteristics support the application of advanced breeding programs aimed at enhancing milk yield and overall economic performance, making farm 2 a valuable model for genetic improvement in dairy cattle.

CONCLUSIONS

Following the completion of this study, several conclusions can be drawn:

- Farm 2 demonstrates superior genetic potential and milk production performance compared to farm 1 across all ancestral lines (mothers, paternal grandmothers, maternal grandmothers), with consistently higher average yields and milk component levels. This reflects not only enhanced genetic value but also more effective herd management and selection strategies.
- The greater genetic variability observed in farm 2, especially through higher coefficients of variation and standard deviations, provides a strong basis for targeted breeding programs. This diversity increases the potential for selecting high-performing individuals and accelerating genetic gain through modern methods.
- The statistically significant difference in milk fat content for maternal grandmothers (0.67 % higher in farm 2, $p < 0.01$) indicates a distinct advantage in milk quality traits in the maternal lineage of farm 2, suggesting opportunities for improving energy-rich milk production and downstream dairy processing value.
- The uniform fat percentages among paternal grandmothers across both farms suggest a stable genetic background for this trait, making maternal lines a more critical factor in future selection programs aiming to enhance milk quality.

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