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ASSESSMENT OF GREY STEPPE CATTLE GENETIC AND PHENOTYPIC TRAITS AS VALUABLE RESOURCES IN PRESERVING BIODIVERSITY

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Abstract

The paper reveals original findings in agrobiodiversity, as part of the global biodiversity puzzle. The Grey Steppe cattle are endangered to extinction and are part of a genetic preservation program in Romania. The research approached the productive performance, the genetic determinism of milk production traits, studied on a Grey Steppe breed population nucleus of 30 individuals, throughout 4 productive cycles. The average milk yield in all 4 consecutive lactations reached 1858 kg per capita, while total lipids and proteins reached 4.41% and 3.56%. Recalculated for Maturity Equivalent, the production summed up 2197 kg milk (4.36% fat, 3.52% proteins). The electrophoresis profiling revealed 6 loci codifying the major proteins in milk: alphacasein S₁ (α S1-cz), beta-casein (β -cz), kappa-casein (K-cz), beta-lactoglobulin (β -lg), alpha-lactoglobulin (α -la) and alpha-casein S₂ (α S2-cz). Apart from these, we identified a new allele in heterozygote state in two individuals of the studied population and we coded it α S1-CN I^{RV}. The main lactoprotein is the kappa-casein (K-cz), identified with high heritability in the studied population ($h^2 = 0.57$ %), while the α S₁ casein (α S₁-cz) had intermediate capacity of transmittance across generations ($h^2 = 0.29$ %). The Grey Steppe breed represents a valuable gene pool due to its high capacity of adaptation to local conditions, to its versatileness in using lower quality feed than other specialized breeds, as well as to its reproductive robustness and to its potential in bearing ancestral, undiscovered genes coding beneficial lactoprotein species.

Key words: biodiversity, genes, Grey Steppe cattle, lactoproteins, milk yield

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1. Introduction

Preservation of biodiversity became one of the most important and urgent challenges of the mankind. Reduction of biodiversity disturbs the fine balances of the habitats, leading to dramatic changes on short, medium and long term, including the extinction of certain life forms within it (GEO, 2005). Perturbation of biocoenosis/ecosystem by certain stressful factors (natural disasters, unsustainable agriculture, industry, tourism) leads to a reduced level of biodiversity, imposing concrete measures of preservation and protection (GEO, 2005; Grec, 2016; Toniuc and Boşcaiu, 1991). One of the ways to preserve biodiversity is to establish and to manage protected areas. However, in most cases, the non-endangered species multiplied better than the real endangered ones in such areas (Otegui et al., 2012). Another way to address biodiversity issues is to rehabilitate certain injured environments, in terms of natural resources control, waste management and monitoring of ecosystems interactions (Isopescu et al., 2012). Romania became part of certain conventions and international agreements dedicated to environment protection, such as the Convention on Biological Diversity (Antofie et al., 2010). According to its

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strategic plan, there are 5 main goals to be accomplished by 2020. One of these is *"To improve the status of biodiversity by safeguarding ecosystems, species and genetic diversity"* (UN, 2017).

Agrobiodiversity is an essential part of the world's biodiversity. More than 75% of the agri-food production in the world is yielded by 25 vegetal and animal species. Management of these species' genetic resources is indispensable and approaches the food security threats. Identification, multiplication. preservation and further development of both vegetal and animal genetic material lead to high productive levels and to the best acclimatization to local conditions, as well. Preserving the agricultural biodiversity is one of the sustainable agriculture priorities (Robu et al., 2009). One of the ways used by the European Union to financially assist the biodiversity protection in anthropic ecosystems, including in farmlands, is the usage of the Agri-Environment Schemes, which support farmers' activity and prevent the uncontrolled multiplication of the herds, necessary otherwise to render economic reliability to the farm but endangering natural biodiversity (Jitea and Arion, 2015).

The amount of domestic animal's species available for farming is around 40, out of which 14 species ensure 90% of the overall animal production (cattle, horses, swine, sheep and goats, rabbits, laying fowl and poultry, fishes). Out of 3882 identified pure breeds, 880 do not have a constant and well-known flock size, are endangered and their genetic pattern There are a few arguments could disappear. supporting the preservation of the animal populations endangered by extinction (Bostan, 2016; Maciuc et al., 2010a): (a) genetic arguments: the endangered populations are a pool of neutral (not active, sleeping) genes in present but potentially useful in the future, to provide reorientation of the productions in relation with the further humans needs, in terms of production levels. bio resistance, production quality, pharmaceutical aspects etc.; (b) zootechnical arguments: such breeds possess certain interesting aptitudes in particular conjunctures (producing of niche type products); (c) ecological arguments: local populations are an excellent study material to better know the phenomenon involved in the genotypeenvironment interactions; (d) cultural arguments: local breeds or varieties are part of common heritage an patrimony, that must be passed along the human generations.

Certain animal species or breeds are superficially evaluated like having poor economic value. Apart from this low yielding potential, the Grey Steppe breed is known for better adaptation to local conditions and particular microbiota, for its better reproductive traits (including easy, unsupervised calving) and for its ability to convert low quality roughages into milk (Metera et al., 2010). If just one of such local species or breeds becomes extinct (prior to its discovery or in-deep knowledge), it could become an irremediable loss for the global bioeconomy, even if the majority of other biological entities and taxa would be well preserved.

Agricola Cardaş, within the paper entitled "Notions on the cattle knowledge and husbandry" (1948) underlined that, between 590-616 A.D., Agiluli, the king of Longobards (settled within alpine areas) received as gift from the Avars tribes, migrated from Urals and Altai steppes, "grey steppe cattle with very long horns". The Balkans peninsula was completely "invaded" by the primigenius steppe cattle type, the ancestor of many cattle breeds from southester Europe and from Romania, as well (Cardas, 1948). The Grey Steppe cattle breed is one of the oldest indigenous breeds, issued from the wild Bos taurus primigenius populations, formed and adapted under the pedo-climatic conditions of the Romanian territory. In the past, the wild species, namely the Aurochs (Bour in Romanian) was spread all across the historical Romanian provinces - Transylvania, Moldavia and Valachia. Until 1850, the cattle herd of the Romanian provinces comprised two main autochthonous breeds, such as Grey Steppe and Romanian Mountain Cow (Mocănița) (Porter et al., 2016), the former one bred within steppe-plain areas and the latter one within mountainous-hilly areas. The environment conditions strongly influenced the synthesis of these local breeds, while the human intervention towards them was minor, hence the lack of modifications in breeds morphology and yielding traits. The first law on the improvement of domestic animals breeds was published in 1892 and the main consequence is the import of individuals from better performance breeds. From that very moment, the genetic structure of the indigenous cattle populations began to modify due to the occurrence of many interbreeding crosses. This is why the actual need of charting the actual herd size of Grey Steppe breed and of its performances, as preliminary work in developing genetic preservation programs dedicated to biodiversity protection in natural and anthropic ecosystems (Maciuc et al., 2010a).

Usage of local breeds, such as Grey Steppe, could contribute to indirectly improve biodiversity of wild flora from pastures and meadows areas, through rational grazing (suppression of harmful vegetal species and supporting of the beneficial one) (Török et al., 2014).

Within this context, this article presents an analysis on the genetic parameters of a local population of cattle (Romanian Grey Steppe), preserved for its inner richness of genes potentially useful in consolidating the adaptation of modern cattle production breeds to adverse environmental conditions, including climate change and disease like threats.

2. Material and methods

According to the F.A.O., the Grey Steppe cattle breed that we addressed in our research is included within the group of populations endangered by extinction (group E), which need "in situ" preservation programs, in order to keep unaltered its genetic structure (FAOUN, 1992). In terms of farming technology, the "in situ" preservation of endangered populations deals with the reproduction and inbreeding of the genetically stable animal bloodlines (Maciuc et al., 2010a; 2011).

The research comprised 30 cows in genetic preservation, Grey Steppe breed, reared within the semi-intensive system, under the conditions provided by the Research and Development Station for Cattle Husbandry, Dancu, Iaşi, Romania. Data were taken from direct observations and laboratory assessments, as well as from the database managed by the Office of Genetic Improvement and Reproduction, Iaşi. There were studied the production traits (milk yield, protein yield, fat yield, percentages of fat and proteins in milk, recorded daily), certain reproduction traits (age at first calving, calving interval) along with the quantitative genetics elements related to certain traits and especially to the milk protein systems (heritability, additive genes variance).

All data were statistically processed to obtain the main descriptors (average, standard mean error, standard deviation, variation coefficient (Petrie and Watson, 2013), while the R.E.M.L. (Restricted Maximum Likelihood) (Mazza et al., 2016) was used to obtain the main genetic parameters. The method is based on a function maximizing iterative process. The calculus varies upon the chosen optimizing algorithm but any version requires BLUP solutions for any iteration cycle, especially when multiple factors are part of the model. A high amount of iterations is required in order to reach convergence if a realistic and effective assessment is desired. Usually, in order to be accepted, the convergence is realized when the difference between the solutions achieved during the last and the penultimate iteration is just one percent lower than the averages of the studied trait (Gengler et al., 1999; Maciuc et al., 2010b; VanRaden and Wiggans, 1991). In this study particular situation, the final convergence was 99.99 %, corresponding to 1687 iterations.

The biometric model we used is presented in Eq. (1) (Kawamoto et al., 1992; Ng-Kwai-Hang Hayes et al., 1986). This is a mixed model, hence it comprises a random variable (the animal) and two fixed variable (the farm and the rank of lactation).

$$J_{ijkl} = \mu + F_i + L_J + A_k + e_{ijkl} \tag{1}$$

whereas:

 J_{ijkl} = performance "*l*" of the individual "*k*", achieved during lactation "*j*", within the farm "*i*",

 μ = general average,

 F_i = effect of farm "*i*" (fixed variable); *i*=1.

 L_j = effect of lactation rank "*j*" (fixed variable); *j*=1. A_k = genetic additive effect of the individual "*k*" (randomized variable).

 e_{ijkl} = error associated to each measured performance.

The PCR-RFLP method (Ngu et al., 2015) was used to determine the genotype at milk proteins loci, while the isoelectric focusing technique (IEF) (Murgiano et al., 2013) was used to study the polymorphism of all lactoproteins in cattle. The milk samples in Grey Steppe cows were taken from every lactating female, using 15 ml Falcon tubes, transported at 4°C then frozen at -20°C till the analysis were carried on. Thawing was run slowly and passively, at laboratory room temperature, then the samples were submitted to centrifugation at 8000 rpm, for 5 minutes, in order to facilitate lipids separations from the lactoserum. Then, they were stored 30 minutes at 4°C, to allow the lipids to solidify then to remove them from each tube using a spatula (Bâlteanu et al., 2007; Creangă et al., 2013a). În order to optimally express the protein concentration, the samples were diluted with an urea and β -mercaptoethanol reagent. The samples were submitted to migration in polyacrylamide gel, 4%. After migration, the gel was immersed in a solution of 10% de trichloracetic acid and the staining was made throughout 2 hours, using a 0.025% Coomassie Brilliant Blue R-250 in 40% ethanol and 7% glacial acetic acid (Bâlteanu et al., 2007; Creangă et al., 2013a, 2013b).

3. Results and discussion

The milk yield, milk quality traits as well as the reproduction indices are revealed in Table 1, as extract of the National Production Official Inspection applied on the studied Grey Steppe herd. The average milk yield for all lactations reached 1858 kg, while the milk quality traits were calculated at 4.41% total lipids and 3.56% proteins. As reported to Maturity Equivalent, the average values reached a yield of 2197 Kg milk per lactation, containing 4.36% total lipids and 3.52% proteins. During the first lactation it has been reached 59.85 % of the maximal lactation, value that depicts the tardiness of the Grey Steppe breed for the milk yield (Chelmu et al., 2013). Total lipids and proteins yields presented quite similar dynamics with that of the milk yield, due to the strong correlation between these traits (rpg = 0.75-0.99%). It must be noticed the high variability of the assessed traits, suggesting strong heterogeneity of the studied population.

The Grey Steppe breed provides high quality productions, especially related to fat and proteins proportions, strongly correlated (75-99%, highly significant for p<0.001). Such qualities, as well as the high heterogeneity suggest the possibility to preserve *in situ* and use so many valuable individuals that could warrant high quality yields, meeting food safety requirements and also providing a selection pool if further biodiversity threats are taken into account. Also, it is known that many local genotypes (including cattle breeds) could become more productive through well managed and appropriate selection programs, without losing their local adaptation features (Hall and Bradley, 1995).

Lactation rank	Females in lactation	Milk yield (kg)	Total lipids (fat)	Proteins		Age at first parturition (months)		Calving interval (days)
			kg	%	kg	%		-
1	9	1315	61.4	4.67	48.1	3.66	28	-
2	9	2476	112.2	4.53	92.6	3.74		426
3	9	2103	86.6	4.12	70.7	3.36		526
4	3	902	39.9	4.42	32.1	3.56		428
Average	30	1858	81.9	4.41	66.1	3.56		483
Average (Maturity Equivalent)	30	2197	95.8	4.36	77.3	3.52		

Table 1. Productive and reproductive traits in Grey Steppe breed, assessed on consecutive lactations

The genetic structure of the milk proteins polymorph systems, such as alpha-casein $S_1 (\alpha S_1-cz)$, beta-casein (β -cz), kappa-casein (K-cz), beta-lactoglobulin (β -lg), alfa-lactoglobulin (α -la) and alpha-casein $S_2 (\alpha S_2-cz)$ is presented in Table 2.

The analysis of electrophoretic profiles revealed 6 loci coding the 6 main protein species in milk through certain common genes as well as through some less frequent ones more difficult to identify via PCR. In the αS1-CN locus, the common B & C alleles have been identified. In two individuals, however, it was found a new genetic variant. This appeared as a main band with its isoelectric point between alleles B & C, closer to the C band. The experiment was triplicated and the same electrophoresis migration behaviour has been noticed. This possible new allele was found in heterozygote state in two individuals and has been code named aS1-CN IRV (Bâlteanu et al., 2007; Creangă et al., 2013a, 2013b). The "I" name has been given in accordance with the nomenclature used in naming the genes coding the main protein systems in milk. Accordingly, any newfc1 genetic version must be named by capital letters of the Latin alphabet. The last discovered version in cattle was "H" (Mahé et al., 1999); the superscript RV was used in order to underline that this genetic version occurred and belongs to an autochthonous breed (RV stands for Romanian Variety) (Bâlteanu et al., 2007; Maciuc et al., 2010a). This profile seems to be similar to the one described in: African Kuri and Sudanese Fulani breeds (Mahé et al., 1999), in Nepalese Bos taurus individuals and Bos taurus X Bos grunniens hybrids (Kawamoto et al., 1992), in northern Eurasia and near Eastern cattle (Meng-Hua et al., 2007). The occurrence of this new allele on the α S1-CN locus has not been previously confirmed by other authors running molecular studies. Consequently, we supposed that the lack of a coherent national breeding program for this cattle breed still hides precious

alleles, yet undiscovered. This state of facts favored the Grey steppe breed in preserving, unaltered by anthropic intervention, many rusticity traits and a high degree of genetic variability.

Studies of quantitative genetics applied on cattle milk production revealed that both casein and serum proteins typology bear a strong genetic determinism and their versions could influence, either in a positive or negative manner, the total protein content of the milk (Maciuc et al., 2010a, 2010b, 2011). Starting from the phenotypic values and the total variance for the main milk proteins in Grey Steppe cattle breed, we computed the genetic variance as well as the heritability coefficients for each trait and lactoprotein. The results are displayed in Table 3.

The heritability coefficient for kappa-casein (K-cz) expresses a stronger degree of hereditary influence, hence the warranty that the phenotypic values correspond in a greater proportion to the constitutive genetic potentials. In this particular situation, the environment is involved in a reduced proportion in determining the total variance. This allows designing different managerial approaches in the genetic improvement works related to the lactoproteins with low genetic transmittance.

Higher "intragroup" variance of β -casein (β -cz) and of β lactoglobulin (β -lg) and the lower variance due to the additive genes induced lower values of the total genetic variance and, consequently, of the heritability coefficients for the incriminated lactoproteins. The low genetic determinism rate of these lactoproteins depicts the high variability of the females that gave the milk samples and, on the other hand, the low genetic variance among the sires. The influence of the environment on determining the variance of such traits is higher. This put these lactoproteins within the weak hereditary transmittance group. An intermediate status has the α S₁ casein (α S₁-cz), whose heredity is average (h² = 0.29%).

 Table 2. Genetic polymorphism of milk proteins in Grey Steppe breed

Frequency	a S1-cz	β-cz	K-cz	B-lg	a-la	a S2-cz
Genotypes frequency	BB = 0.5 BC = 0.3 CIRV = 0.1 BIRV = 0.1	$\begin{array}{c} A_1 A_1 \!=\! 0.2 \\ A_1 A_2 \!=\! 0.5 \\ A_2 A_2 \!=\! 0.3 \end{array}$	AA = 0.209 AB = 0.416 BB = 0.375	AA = 0.292 AB = 0.500 BB = 0.208	BB = 1	AA = 1
Alleles frequency	$p_{\rm B} = 0.7$ $q_{\rm C} = 0.2$ $r_{\rm IRV} = 0.1$	$\begin{array}{l} p_{A1} = 0.45 \\ q_{A2} = 0.55 \end{array}$	$\begin{array}{c} p_{A1} = 0.417 \\ q_{A2} = 0.583 \end{array}$	$\begin{array}{l} p_{A1} = 0.542 \\ q_{A2} = 0.458 \end{array}$	$P_{B} = 1$	$p_A = 1$

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Trait	Heritability	Additive genes variance	Intragroup variance	Total variance
Length of normal lactation (days)	0.24	1799.038	4628.290	6427.329
Milk Yield (Kg)	0.26	46247.036	340772.720	387019.760
Total lipids (%)	0.71	0.9212	0.675	1.596
Fat (Kg)	0.29	166.581	614.559	781.140
Proteins (%)	0.63	0.908	0.701	1.7089
Proteins (kg)	0.27	203.198	479.966	683.164
K_cz (%)	0.57	0.204	0.3	0.504
β_cz (%)	0.21	0.048	0.8	0.848
β_lg (%)	0.19	0.132	0.3	0.168
αS1- cz (%)	0.29	0.696	1.8	1.104

Table 3. Heritability of the main productive traits and protein systems in Grey Steppe milk

The main milk lactoprotein, i.e. the kappacasein (K-cz), well known in literature for its high degree of hereditary transmittance was confirmed to have the same high heritability ($h^2 = 0.57\%$) on the Grey Steppe studied population. The same lactoprotein, the kappa-casein (K-cz), has a positive dynamics, intense correlated with the fat percentage in milk (Vanbergue et al., 2016). This latter trait is high heritable and strongly correlated (> 45%) with the protein percentage in milk (P<0.05) (Table 4 and Fig. 1). The diagram of regression curve between K-cz (kappa-casein) and fat percentage in milk indicates a straight proportional reciprocal dynamic: the richer in lipids, the richer in kappa-case in the milk will be. The points certifying the strong correlation between the analyzed traits were found close or overlapped on the regression line. Despite the fact that the alleles identified in our research could induce lower development of the udder leading thus to lower milk yield, the milk quality will be better.

Basing on the heritability coefficients of the main production traits as well as of the lactoproteins specificity in the Grey Steppe cattle, it could be assessed the dynamics of the selection effects, which are in accordance with the genetic improvement and preservation objectives applied to the studied herd which have high genetic patrimonial value for Romania (Köhn et al., 2009, Maciuc et al., 2010a; Ng-Kwai-Hang Hayes et al., 1986; Wildman et al., 1982).

One of the main issues in the heredity of quantitative traits is to know up to which extent the traits within certain pairs are or are not interrelated. This state is tested through the computation of correlation coefficients. The analyzed traits, paired differently, revealed multiple values and significations for the phenotypical and genotypical correlations (Table 5). Among many traits pairs we studied, it must be noticed the positive and strong phenotypical and genotypical correlations between milk yield and fat yield in milk ($r_{pg} = 0.98-0.97$) the same pair and situation being also identified in other research (Montaldo et al., 2015). Same positive correlation trend, although average as intensity was identified between the milk yield and the body development traits ($r_{pg} = 0.33 - 0.44$), quite similar with the findings of Bilal et al., (2016) on Holstein cows.

		Standa	rdized coefficients	Regression coefficients	t	Sig.	
		В	Std. Error	Beta			
1	(Constant)	.884	1.667	103	.531	.049	
1	ln1g	.588	.392	.493	1.502	.050	
a da	a dependent with the variable: K oz (kanna casein) la la total linids (fat) %						

Table 4. Regression coefficient between K-cz and total lipids (fat) %

a dependent with the variable: K-cz (– kappa casein); ln1g – total lipids (fat) %

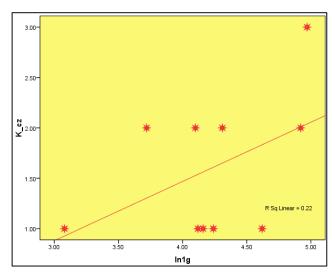


Fig. 1. Regression line between K-cz (kappa-casein) and ln1g (fat %)

Correlated traits	$r_{p}\pm$	Srp	$r_g \pm$	Srg
	Milk yield versus:			
Length of gestation	0.18	0.07	0.25	0.04
Length of lactation	-0.25	0.03	-0.26	0.05
Fat percentage in milk	-0.21	0.02	-0.19	0.01
Fat yield in milk	0.98	0.01	0.97	0.03
Withers height	0.38	0.06	0.33	0.01
Chest circumference	0.39	0.06	0.32	0.01
Live weight	0.44	0.06	0.41	0.01
D	Duration of gestation vers	sus:		
Fat percentage in milk	0.09	0.08	0.13	0.06
Fat yield in milk	0.07	0.08	0.10	0.07
Withers height	0.28	0.07	0.43	0.09
Thoracic perimeter	-0.11	0.01	-0.13	0.05
Live weight	-0.10	0.07	-0.30	0.04

Table 5. Phenotypical (r_p) and genotypical (r_g) correlations between the main morphological-productivetraits in Grey Steppe Cattle Breed

The classical correlation between the milk yield and the fat percentage in milk was found as negative (-0.21, -0.19), knowing the mammary gland transfers less lipids per unit of milk produced, as the intensity of the yield is higher (Jattawa et al., 2016). The correlations between the duration of gestation and certain production and body score traits were rather weak, either positive or negative (Ng-Kwai-Hang Hayes et al., 1986; Wildman et al., 1982).

Investigation of such correlations between milk production traits helped us to better understand their intensity level and better explain their genetic determinism, knowing the studied breed is a rustic one and humans din not interfere significantly with its synthesis and evolution. In Grey Steppe breed, the lactation lasts just 7-8 months, while gestation length is 14.3% shorter than usual. Hence environmental factors strongly affected the breed dynamics and the gestation lasts around 240 days (compared to 280 days - average of the species), the main correlations involving milk yield and body development had low values and were not significant, comparing with genetically improved breeds, in which same correlations have high and significant values (Bilal et al., 2016; Řehák et al., 2012; Tsuruta et al., 2005).

The achieved results could serve in the safeguard of the endangered Grey Steppe breed, which has its importance in preserving biodiversity and keeping ancestral genes available for future challenges when their potential could become more beneficial for surviving of the species or even to mankind welfare, despite the fact that such a breed could be considered, accordingly to modern animal production records, as a low performing breed. This breed could play a historic, touristic and genetic role in the near future, due to its specific traits, such as: high content of lipids in milk, high rusticity level, high resilience to diseases and unfavorable environment conditions, good adaptability to poor or traditional farming conditions.

4. Conclusions

The new allele for alpha casein, code named α S1-CN I^{RV}, was found in heterozygote state in two

individuals, indicating that the Grey Steppe breed still hides precious alleles, yet undiscovered. The genotype of the breed is unaltered by human intervention, preserving many rusticity traits and a high degree of genetic variability, indicating strong genetic diversity level.

High variability of the studied population was identified for total lipids and proteins yields that presented quite similar dynamics with that of the milk yield, while the correlation between these traits was also high ($r_{pg} = 0.75 - 0.99$ %). Such qualities, as well as the strong heterogeneity suggest the possibility to preserve *in situ* the breed and to use valuable individuals that could warrant high quality yields, meeting food safety requirements and provide a selection pool if further biodiversity threats are taken into account.

Extrapolating from the results related to heritability and correlations of milk yield, protein yield and fat yield, as well as from the correlations occurred between kappa casein and total lipids yield, it could be stated that some individuals in the analysed population could be used as valuable genes disseminator in crossing with the local populations of cattle, in order to make them able to produce milk with better cheese-making qualities (rich in digestible proteins and with potential in developing specific sensorial traits such as particular tastes and flavours, especially in maturated cheese).

More in-deep research is necessary to continue such investigations, using modern biomolecular techniques and biotechnologies, in order to bring at its best the understanding of the genetic potential the Romanian Grey Steppe cattle breed. Our research is far from being complete but it belongs to the scientific community efforts to protect endangered species or breeds in order to preserve biodiversity within a more and more human controlled and transformed environment.

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