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HIGHLY EFFECTIVE METHOD OF DNA EXTRACTION FROM BLOOD: A FIRST STEP FOR ANALYSIS OF GENETIC DIVERSITY OF INDIGENOUS CATTLE BREEDS

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Abstract

In molecular genetics analysis, isolation and quantification of DNA represents a step extremely important. DNA isolation methods are based on purity, integrity and the amount of DNA obtained. The degree of DNA purity is one of the most important factors in the reproducibility of the PCR method. DNA is considered pure if the ratio of the two spectrophotometric readings, A260/A280, shows values between 1.7 - 2.0. Values lower than 1.7 indicate impurities with proteins, and a ratio higher than 2.0 indicates impurities with other contaminants. The aim of this research was to evaluate the effectiveness of the automatic method of DNA extraction from a number of 24 blood samples collected from Pinzgauer cattle breed, for the analysis of genetic diversity. The amounts of DNA extracted from the 24 blood samples ranged from 13-61.9 ng/µl and the absorbance ratio A260/A280 showed values between 1.61-2.13. The results obtained demonstrated the effectiveness of the automatic method of DNA extraction, and thus, isolated and quantified genetic material can be used further in the next stages of genomic analysis of this breed.

Key words*: cows, DNA quantification, genomic analysis, Pinzgau.*

INTRODUCTION

The problem of preserving the genetic resources, respectively the indigenous cattle breeds, with cultural-historical importance, endangered, represents a topic of major importance.

The Pinzgau breed has a major importance in the livestock sector, being adapted for growth and exploitation in areas with altitudes between 400-1600 m, rich in rainfall and fertile natural meadows, being resistant to severe environmental conditions (Feldhamer, 2007).

In the last 15 years, about 300 breeds of approx. 6,000 belonging to different species of farm animals have been identified by the Food and Agriculture Organization of the United Nations (FAO) as endangered. Among cattle breeds, the most endangered are the podolian breeds from different parts of Europe. Podolian cattle belong to a group of very old European breeds, having ancestors in Bos primigenius (Gentry, 2011).

Piera Di Lorenzo et al. (2018) studied the genetic diversity of Podolian breeds and aimed to reconstruct their origin. A number of 18 podolian breeds (Piemontese, Bianca di Val Padana, Romagnola, Mucco Pisano, Calvana, Chianina, Maremmana, Marchigiana, Italian Podolian, Ukrainian Gray, Romanian Gray, Hungarian Gray, Slavonian Syrmian Pod., Istrian) have been studied phylogenetically.

To interpret the results, nine other cattle breeds were compared (Valdostana, Gray Alpine, Italian Brown, Italian Red Pied, Cabannina, Reggiana, Agerolese, Cinisara, Modicana).

The global analysis clearly highlighted some peculiarities of some genes in the mtDNA group. The analysis of the main components indicated a genetic proximity between five breeds (Chianina, Marchigiana, Maremmana, Podolica Italiana and Romagnola).

A suggestive hypothesis shows the ancestral double contribution to the current genealogical background of podolian breeds (Piera Di Lorenzo et al., 2018).

Another type of genetic research focused on the study genetic markers associated with the characteristics of milk/meat production, especially in the case of endangered cattle

breeds, which is useful for assessing the conservation value of genetic resources of animal origin as well as for determining the degree of uniformity of breed (Lewin, 2004).
Many other molecular markers associated with the place of formation and domestication of the breed were analyzed over time by numerous researchers in the field.
The issue of preserving local breeds is important for ensuring the food security of the Romanian population, given the recent climate changes, not only of rising temperatures, but temperature fluctuations and extreme phenomena that could bring new challenges in the future.
A first step in studying the genetic diversity of these breeds is the extraction of DNA from biological samples.
During the process of DNA denaturation, the nitrogenous bases located inside molecule manifests absorption capacity of ultraviolet radiation at $\lambda = 260$ nm, if we compare to the situation when they were arranged inside the double helix (Thomas, 1994).
The hyper chromic effect affects the absorbance ratio of working solutions at wavelengths of 260 and 280 nm (A260/A280), which must be between 1.8 and 2, though some authors allow values between 1.7 and 2.
Lower values suggest higher absorbance and, indirectly, higher protein concentrations in the working samples, which absorb ultraviolet light at 280 nm; higher ratio values indicate RNA contamination.
This paper aims to validate the optimal method of extraction and quantification of total DNA from blood samples collected from the Pinzgau cattle breed, a first step in the analysis of genetic diversity.

MATERIALS AND METHODS

The quantification of the genetic diversity of Pinzgau cattle breed was initiated by the stage of collecting a number of 24 biological samples, respectively blood. Blood samples were collected by jugular vein puncture, using Vacutainer tubes with EDTA to prevent clotting, with a capacity of 2 ml and 18 G collection needles.
DNA extraction from blood samples was performed by the automated method with Maxwell equipment 16. This equipment can process up to 16 samples in 40 minutes, and the extracted DNA can be used in a variety of applications, including PCR and agarose gel electrophoresis.
The amount of total DNA was quantified using a Nanodrop spectrophotometer and the optical density was measured at the absorption rate A260 nm and A280 nm, subsequently making the ratio between the two absorption rates.
Nanodrop is a spectrophotometer, with a measuring spectrum between 220-750 nm, which measures samples with a volume of 1µl, with a very high accuracy and reproducibility. It uses high-performance technology that involves tensioning the surfaces on which the measurement is made to retain the sample.
The results were statistically interpreted using a series of basic indicators, such as the arithmetic mean, standard deviation, standard error and confidence limit of the mean, coefficient of variation.

RESULTS AND DISCUSSIONS

Most biological substances have a characteristic absorption rate in the field of ultraviolet (UV) radiation (Higgins, 1988).
Thus, the absorption rate of 260 nm corresponds to nucleic acids, 280 nm to proteins and 230 nm to various contaminants.
The interpretation of the quantification results is based on the fact that the DNA is considered sufficiently pure, if the ratio of the two readings, respectively A260/A280, has values included in range 1.7-2.0.
Values lower than 1.7 indicate impurities with proteins, and those higher than 2.0 indicate impurities with other contaminants.
Beer Lambert's law shows that there is a linear relationship between the concentration of a compound and its absorbance at a certain wavelength. The calculation of the DNA concentration is based on this fact, but assessments are also made on the purity of the DNA in relation to the proteins (Tamura, 2013).
Regarding the age of the individuals from whom the blood samples were taken, there is a series of information that shows that the age range for the 24 females was between 12-90 months, with an average of 52 months.

As can be seen in figure 1, a number of 11 females are 5-10 years old, 8 are age limits between 2-5 years and 5 are in the range of 1-2 years (Table 1 and Figure 1).

Table 1. Identification data of Pinzgau individuals from which biological material was collected for the purpose of genetic analysis

No.	Cattle breed	Age (months)
1.	Pinzgau	16
2.	Pinzgau	83
3.	Pinzgau	72
4.	Pinzgau	65
5.	Pinzgau	60
6.	Pinzgau	33
7.	Pinzgau	58
8.	Pinzgau	64
9.	Pinzgau	23
10.	Pinzgau	34
11.	Pinzgau	33
12.	Pinzgau	90
14.	Pinzgau	86
15.	Pinzgau	84
16.	Pinzgau	81
17.	Pinzgau	66
18.	Pinzgau	66
19.	Pinzgau	50
20.	Pinzgau	63
21.	Pinzgau	12
22.	Pinzgau	20
23.	Pinzgau	18
24.	Pinzgau	18

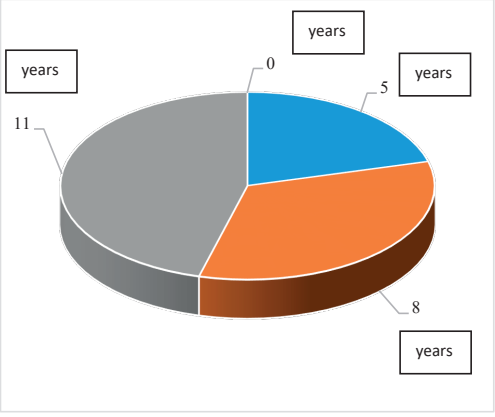

Figure 1. Numerical distribution by age of Pinzgau cattle breed

Following the quantification of DNA samples extracted from the 24 females, the concentrations varied in the range of 4.1-61.9 ng/µl, the average concentrations being 27.7 ng/µl (Table 2) and the ratio of the two absorptions, respectively A260/A280, presented values in the range 0.68-2.13, with an average of 1.68.

Table 2. Results of spectrophotometric quantification of total DNA extracted from blood samples from the Pinzgau cattle breed

ID Sample	Abs260	Abs280	Abs230	260/280	260/230	Concentration DNA (ng/µl)
P_01	0.352	0.218	0.375	1.61	0.94	17.6
P_02	0.447	0.289	0.480	1.55	0.93	22.3
P_03	1.24	0.842	1.212	1.47	1.02	61.9
P_04	0.369	0.230	0.339	1.60	1.09	18.4
P_05	0.486	0.305	0.430	1.59	1.13	24.3
P_06	0.844	0.500	0.661	1.69	1.28	42.2
P_07	0.371	0.218	0.382	1.70	0.97	18.5
P_08	0.385	0.227	0.373	1.70	1.03	19.2
P_09	0.589	0.369	0.627	1.60	0.94	29.4
P_10	0.796	0.506	1.024	1.57	0.78	39.7
P_11	0.404	0.266	0.409	1.52	0.99	20.2
P_12	0.603	0.377	0.800	1.60	0.75	30.1
P_13	0.821	0.488	0.559	1.68	1.47	41.0
P_14	0.922	0.526	0.579	1.75	1.59	46.0
P_15	0.959	0.570	0.666	1.68	1.44	47.9
P_16	0.547	0.315	0.486	1.74	1.13	27.3
P_17	0.347	0.168	0.311	2.07	1.12	17.3
P_18	0.355	0.171	0.301	2.08	1.18	17.7
P_19	0.262	0.147	0.241	1.78	1.09	13.0
P_20	0.084	0.123	0.280	0.68	0.30	4.10
P_21	0.306	0.169	0.400	1.81	0.76	15.2
P_22	0.398	0.187	0.226	2.13	1.76	19.9
P_23	0.627	0.299	0.557	2.10	1.13	31.3
P_24	0.806	0.468	0.826	1.72	0.98	40.3

Figure 2 shows the DNA concentration values that were in the range of 4.1-61.9 ng/µl and an average of 27.7 ng/µl.

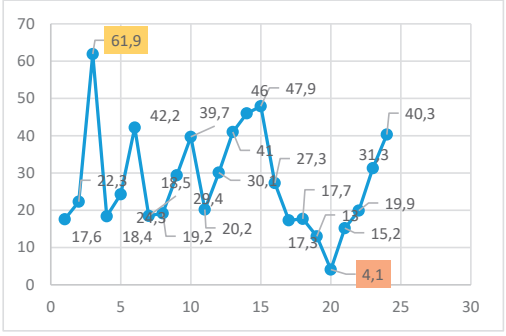

Figure 2. DNA concentration values (ng/µl)

A comparison between the minimum and maximum values, respectively the average value of the data obtained on the concentration of quantified DNA can be seen in Figure 3.

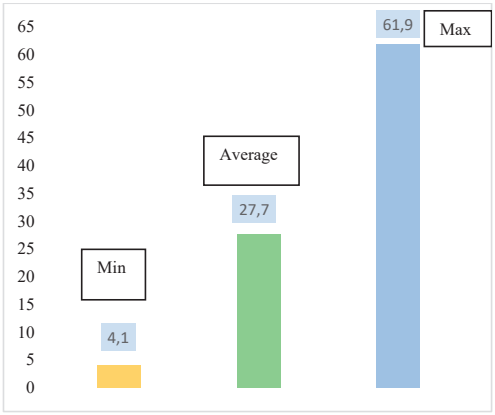

Figure 3. Comparison between the mean value of the DNA concentration, in relation to the minimum / maximum values (ng/µl)

The results of the quantification analysis demonstrated the effectiveness of the DNA extraction method (Figure 3).
Another important aspect in molecular genetics analyzes concerns the purity of the extracted DNA which is evaluated on the basis of the A260 / A280 absorbance ratio. The values obtained are usually classified into 3 ranges (<1.7; 1.7-2.0; > 2.0).
The percentage distribution of the 24 samples, depending on the value of the absorbent ratio A260 / A280 is represented in Figure 4.

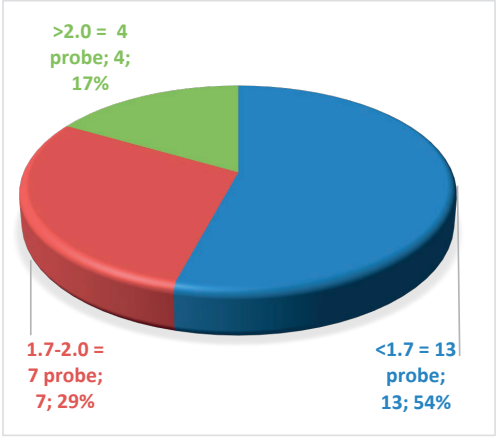

Figure 4. Percentage distribution of samples according to the value of the absorbent ratio A260/A280

Out of the total of 24 samples, 13 presented a value lower than 1.7, a number of 7 samples had values in the range 1.7-2.0 and 4 samples had values higher than 2.0.

A statistical expression of the values obtained for the DNA concentration can be consulted in Table 3, which results in a value of the confidence limit of 5.75 and represents the confidence interval that demonstrates that the calculated parameters are in the desired range.

Table 3. Statistical calculation of values associated with DNA concentration

Statistical parameters	DNA concentration (ng/µl)
Minimum value	4.1
Maximum value	61.9
Average	27.7
Standard deviation	13.61
Standard error	5.55
Confidence limit	5.75

In this case, a homogeneity of the data can be observed, not recording aberrant values compared to the average results of DNA sample concentrations which is 27.7 (ng/µl). The values obtained from the spectrophotometric analysis are found around the average.

CONCLUSIONS

Molecular analysis techniques have evolved considerably over time, allowing the investigation of genome diversity by sequencing it, in different species of zootechnical interest. Thus, the phylogeny of many animal species has been studied by researchers since 1980 (Wellmann, 2019; Wheeler, 2011).
Research in the past has been based mainly on the study of genetic markers associated with characters in individuals of different species of interest, as well as research on microsatellites due to the very high polymorphism and the large amount of genetic information.
The results of the investigations regarding the analysis of the total DNA of the Romanian Pinzgau cattle breed, led to the formulation of the conclusion that the spectrophotometric quantification of the totally isolated DNA validated quantitatively and qualitatively the stage of isolation and purification of nucleic acids, making possible the transition to the next stages of analysis.
Thus, the use and testing of new molecular marker research techniques will help clarify issues regarding the genetic diversity of species of interest.

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