ANALYSIS OF THE PURITY OF DNA ISOLATED FROM BLOOD SAMPLES AT PINZGAU CATTLE

M.A. Davidescu[1*], M. Ivancia[1], D. Simeanu[1], Ș. Creangă[1]

[1]Faculty of Food and Animal Sciences, Iasi University of Life Sciences, Romania

Abstract

There is an increasing interest in the genetic quantification of bovine DNA. Analysis of genetic diversity using DNA extracted from the blood is affected by the quality and the quantity of the DNA extracts, which are critical factors that limit the accuracy and sensitivity of molecular studies. The purpose of this paper is to show the quantity and purity of DNA isolated from a number of 24 blood samples from Pinzgau cattle, using two basic techniques, the automatic DNA extraction technique, using Maxwell™ 16 and 16 MDx instruments and the spectrophotometric quantification technique, using Nanodrop ASP-3700 spectrophotometer. The obtained results are considered satisfactory in terms of the purity of the obtained DNA extracts, 13 samples having the DNA isolate contaminated with proteins based on the absorbance ratio A260/A280 lower than 1.7, the solution in this situation being the repetition of the protein precipitation process.

Key words: *bovines, DNA quantification, DNA purity, spectrophotometry*

INTRODUCTION

In Romania, animal breeding has been an occupation for centuries, the natural environmental conditions, provided by a relief made up of areas included in the Danube Delta, the plains of Banat, Transylvania, with particularly fertile lands, always constituting rich fodder resources for growing in especially cattle and sheep. Cattle breeding has been a concern since ancient times, at first for meat production and work in agriculture and later for milk production (Acatincai, 2004; Acatincai, 2010). In the category of the oldest cattle breeds, there is also the Pinzgau breed, which is of major importance in the livestock sector, being adapted for growth and exploitation in areas with altitudes between 400-1600 meters, rich in precipitation and fertile natural meadows, having increased resistance to diseases and severe climate conditions (Cotos, 2005; Fisteag, 1956; Fisteag et.al., 1958). The Pinzgau breed from Romania is considered to be formed by crosses between Grey Steppe cows and Pinzgauer bulls from Austria (Angelescu, 1974; Mang, 2011). Pinzgau is in endangered cattle breed, the F.A.O. Organization, noting this issue of national interest through the reports issued on the risk status of the breed, which draw attention to the numerical decline. Also, this breed represents a part of our country's history, just as the different varieties of these breeds represent the same thing in the countries where they were formed. The methods applied worldwide, in order to conserve her genetic resources, have evolved considerably over time and the reported results are interesting from the point of view of the phylogeny and genetic evolution of the breed (Scherf, 2000). The deoxyribonucleic acid (DNA) molecule, formed by the polymerization of monomeric units named deoxyribonucleotides, provides informational support for storing, inheriting, and expressing of genetic information in protein molecules. Its double-stranded, helical, and antiparallel structure, with the complementarity between nitrogenous bases A=T, G≡C, was described in 1953 by James Watson (1928-) and Francis Crick (1916-2004) in the paper entitled

*Corresponding author:
mada.davidescu@gmail.com
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"Molecular structure of Nucleic Acids – A structure for Deoxyribose Nucleic Acids" published in Nature journal, vol. 171, no. 4356, pp. 737-738 (Davidescu et. al, 2020a). The quality and purity of DNA is an extremely important aspect for obtaining valid results in the genetic analysis of cattle. The purpose of this paper is to analyze the genetic purity of the DNA isolated from the blood samples, collected from the Pinzgau breed, results that will be useful in the analysis of the genetic diversity of the breed and also in the development of national programs for the conservation of genetic resources.

MATERIAL AND METHODS

The analysis of DNA purity of Pinzgau cattle breed, had as the first step, collection the biological samples, respectively blood, in the case of a number of 24 females in the age category 1-7 years. Samples were collected by puncturing the jugular vein, using anticoagulant tubes for collection (EDTA-ethylene-diamine-tetra-acetic acid). Isolation of DNA from blood samples was performed by the automated method, with the Maxwell™ 16 and 16 MDx instruments, using a special kit provided by the Promega distributor: 48 Maxwell™ 16 MCD LEV, containing 50 LEV purification pistons, 50 of elution tubes, 20 ml lysis buffer, two 1 ml proteinase K solutions and 20 ml elution buffer. 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 DNA extraction protocol comprises several steps: homogenize blood samples for at least 5 minutes at room temperature; preparation and labeling of incubation tubes that must be compatible with the heating block of the extraction equipment; add a volume of 30 µl proteinase K (PK) solution to each incubation tube; add samples (up to a volume of 300 µl) to the incubation tubes; add a volume of 300 µl lysis buffer to each incubation tube; vortex the tubes for 10 seconds; incubate the tubes in the heating block (set at 56°C), 20 minutes, during which the cartridges are prepared; transfer of blood lysate samples from the incubation tube to the cartridges. The method has a number of advantages, including quantitative precision, accuracy, speed, high degree of automation and obtaining DNA samples of good concentration and purity. The amount of total DNA was quantified using the Nanodrop ASP-3700 spectrophotometer. This spectrophotometric method is based on the following principle: most biological substances show a characteristic absorption rate in the ultraviolet (UV) radiation range. Thus, the absorption rate of 260 nm corresponds to DNA/RNA nucleic acids, that of 280 nm to proteins and that of 230 nm to different contaminants (Cojocaru et al., 2004; Rapp, 2010). In this study, the optical density was measured at the absorption rate A260 nm and A280 nm, then making the ratio between the two absorption rates.

RESULTS AND DISCUSSIONS

Most biological substances have a characteristic absorption rate in the range of ultraviolet radiation. In the case of nucleic acids, it corresponds to an absorption rate with a wavelength $\lambda = 260$ nm and in the case of proteins, $\lambda = 280$ nm. The quantification of the DNA samples belonging to the 24 cows varied in the range of 4.1-61.9 ng/µl, the average being 27.7 ng/µl (Table 1 and Figure 1) and the ratio of the two absorptions, respectively A260/A280, presented values in the range of 0.68 - 2.13, with an average of 1.68.

Table 1. Spectrophotometric quantification of total DNA extracted from blood samples

Sample	Abs260	Abs280	Abs230	260/280	260/230	DNA concentration (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

P_01..P_24 - no. of. samples
Abs – absorbance

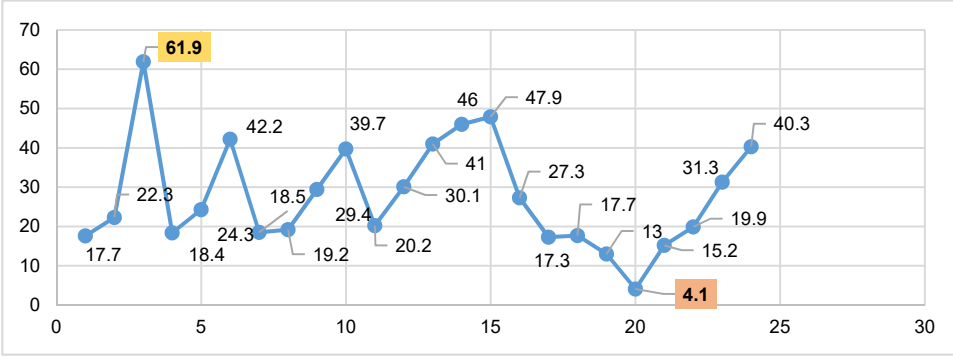
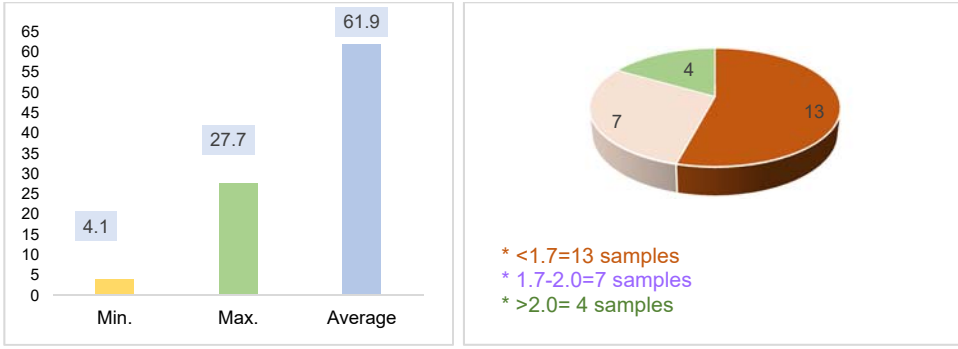


Fig. 1. Extracted DNA concentration values, measured with Nanodrop ASP-3700 spectrophotometer (ng/µl)

Figure 1 shows the DNA concentration values that were in the range of 4.1-61.9 ng/µl. A comparison between the minimum and maximum values, respectively the average value of the data obtained regarding the concentration of quantified DNA can be seen in fig. 2.a.



a. b.

Fig. 2. a. Extracted DNA concentration values, measured with Nanodrop ASP-3700 spectrophotometer (ng/µl); b. Percentage distribution of samples according to the value of the absorbent ratio A260/A280

The results obtained regarding to the quantification of DNA, demonstrated the effectiveness of the DNA extraction method. Another important aspect in molecular genetic analyzes concerns the purity of the extracted DNA which is evaluated based on the A260/A280 absorbance ratio. The values obtained are usually classified in 3 ranges (<1.7; 1.7-2.0; >2.0). The percentage distribution of the 24 samples, depending on the value of the A260/A280 absorbent ratio, is represented in graph 2.b. Out of the total of 24 samples, 13 showed a value lower than 1.7, a number of 7 samples had values in the range of 1.7-2.0 and 4 samples had values higher than 2.0. According to Beer Lambert's law, there is a linear relationship between the concentration of a compound and its absorbance at a given wavelength. The calculation of DNA concentration is based on this fact, making judgments about its purity in relation to proteins (Cojocaru et. al., 2004). The DNA is considered sufficiently pure, if the ratio of the two readings, respectively A260/A280, has values in the range of 1.7-2.0. Values lower than 1.7 indicate impurity with proteins and those greater than 2.0 indicate impurity with other contaminants (Davidescu et. al., 2020b).

CONCLUSIONS

The results that did not fall within the desired range, 1.7-2.0, indicate either an impurity of the samples with insignificant amounts of protein substances or with other contaminants. These situations can occur during the sample preparation stage as a result of improper handling. The results do not deviate much from the normal values, therefore that did not interfere with the next stages of work. However, it is recommended to prepare samples under sterile conditions and also to use sterile equipment and consumables.

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