ASSESSMENT OF DNA PURITY AND QUANTITY IN TELEORMAN BLACK HEAD, SUFFOLK AND ÎLE DE FRANCE SHEEP BREEDS

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Abstract

The increasing demand for sheep meat requires modern methods to improve production, and genetic selection through genetic markers plays an important role in this process. The analysis of genetic markers depends on the quality of the extracted DNA, and assessing its purity through spectrophotometry is essential for the validity of genetic studies. In this study, blood samples were collected from three sheep breeds –Teleorman Black Head, Suffolk, and Ile de France. DNA was manually extracted using the Wizard Genomic DNA Purification kit (Promega), and purity was determined using the Nanodrop ASP-3700 spectrophotometer, ensuring precise evaluation of the A260/A280 ratio and DNA concentration. The DNA purity analysis of blood samples from the three sheep breeds demonstrates satisfactory results for the majority of samples. Out of 34 samples analyzed, 16 exhibited A260/A280 ratios within the ideal range of 1.7 to 2.0, indicating high-quality DNA suitable for further genetic analysis.. These results highlight the overall effectiveness of the DNA extraction process and support the reliability of subsequent genetic studies.

Key words: *DNA purity, spectrophotometry, sheep breeds, Teleorman Black Head*

INTRODUCTION

The discovery of DNA and its applications in molecular sciences have enabled significant advancements in health, research, and animal production [1]. Nevertheless, access to and use of genetic resources remain below expected levels in many developing countries [2] These circumstances highlight the importance of optimizing biotechnological processes and establishing foundational molecular methodologies as tools for economic and social progress.

The quality and purity of DNA play a crucial role in obtaining valid results in molecular techniques such as PCR and genomic sequencing. High DNA purity is essential to prevent contamination that could interfere with data interpretation, while an adequate concentration ensures optimal amplification in subsequent analyses [3]. Therefore, precise measurement of DNA purity and concentration is a fundamental step for the success of these research efforts.The purpose of this study is to analyze the genetic purity of DNA isolated from blood samples collected from three sheep breeds: Teleorman Black Head, Suffolk, and Île-de-France. These results are essential for proceeding with the next stages, which aim to investigate MSTN (myostatin) gene variations in sheep. This research is part of a PhD thesis that will be focused on the genetic determinism of growth in these breeds.

Research has shown that one of the most precise selection methods is marker-based selection [4]. This approach involves specific genes or gene sequences with

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known locations on chromosomes, which can be used to identify an individual or the traits influenced by these genes.

Given the limited studies on this topic in the country, I deemed it necessary to investigate the marker gene myostatin (MSTN), also known as GDF8 (growth and differentiation factor 8). This gene acts as a negative regulator of myogenesis during skeletal muscle development, and its variations have been associated with differences in musculature in certain sheep breeds.

In the process of identifying MSTN gene variations and analyzing their influence on one or more traits, several stages are required. These begin with the careful selection of animals from which samples will be collected, followed by complex genetic extraction procedures, advanced genetic analyses, and the evaluation of traits directly influenced by myostatin gene expression.

Following sample collection, the next important step is DNA purification and quantification, so this study focuses on these aspects in three sheep breeds: Teleorman Black Head, Île-de-France, and Suffolk. The Teleorman Black Head, a native breed, is known for its dual-purpose milk and meat production, showing favorable results in traits associated with meat yield [5]. The other two breeds, Île-de-France and Suffolk, are internationally recognized imported terminal breeds, widely used to enhance body development and carcass quality traits. Ensuring accurate DNA purification and quantification is essential to proceed with further analyses.

MATERIAL AND METHOD

The biological material used in this study consisted of blood collected from three sheep breeds: Teleorman Black Head, Île-de-France, and Suffolk. Blood samples were collected by jugular venipuncture into vacutainers with EDTA, an anticoagulant used to prevent clotting. A total of 34 samples were collected, each individually labeled to facilitate identification and simplify laboratory procedures. Labels for each sample contained the order number, the sex of the animal (M/F), and initials indicating the breed (TBH – Teleorman Black Head, IF – Île-de-France, S – Suffolk).

After collection, which was performed at different times for each breed, the blood samples were stored at -20°C until DNA isolation, with Île-de-France samples stored for seven months and the other two breeds' samples stored for three months. Blood was collected from 14 Teleorman Black Head sheep (9 females and 5 males), 10 Île-de-France sheep (5 females and 5 males), and 10 Suffolk sheep (5 females and 5 males).

The DNA extraction from the sheep blood samples was conducted manually using the Wizard™ Genomic DNA Purification Kit. This kit includes the necessary solutions for the various steps of the process: cell lysis solution, nuclear lysis solution, and protein precipitation solution.

The materials used included vacutainers with K_3EDTA anticoagulant, automatic pipettes and tips (10, 100, and 1000 µl), sterile 1.5 ml Eppendorf tubes, the Wizard Genomic DNA kit, isopropanol, 70% ethanol, a thermoblock, a microcentrifuge, and a vortex mixer.

The DNA extraction method involved several successive steps. In the first stage, cell lysis, 300 µl of blood was transferred to a 1.5 ml Eppendorf tube with 900 µl of cell lysis solution. The mixture was homogenized by inversion and incubated for 10 minutes at room temperature. The samples were then centrifuged at 13,000xG for 20 seconds, and the supernatant was removed, leaving approximately 10 µl of residual liquid above the pellet, which was vortexed for 20 seconds.

In the second stage, nuclear lysis and protein precipitation, 300 µl of nuclear lysis solution was added, and the mixture was pipetted 5–6 times for homogenization. Next, 100 µl of protein precipitation solution

was added, and the mixture was vortexed until small, dark-colored protein precipitates became visible. The mixture was then centrifuged at 13,000xG for 3 minutes to separate the protein residues.

In the third stage, DNA precipitation and rehydration, the resulting supernatant was transferred to another tube containing 300 µl of isopropanol. The tube was gently inverted, allowing the DNA to precipitate as visible white filaments. After centrifuging at 13,000xG for 1 minute, the supernatant was removed, and the DNA pellet was washed with 300 µl of 70% ethanol, followed by another 1-minute centrifugation at the same speed. The ethanol was then aspirated, and the DNA pellet was left to air dry for 10–15 minutes. Finally, the DNA was rehydrated in 50 µl of rehydration solution for 1 hour at 65°C [6].

The concentration and purity of the extracted DNA were determined by spectrophotometry using the NanoDrop ND-2000 spectrophotometer. Absorbance measurements were taken at wavelengths of 260 nm and 280 nm to evaluate both the DNA concentration and its purity.

RESULTS AND DISCUSSIONS

Given the absorption characteristics of nucleic acids, the spectrophotometric analysis of the 34 DNA samples isolated from the three sheep breeds provided data on both the quantity and purity of the extracted DNA (Table 1).

For the Teleorman Black Head breed, DNA concentration values ranged from 10.4 to 86.7 ng/µl, with an average of 30.6 ng/µl (Figure 3a).

For the Île-de-France samples, the average DNA concentration was 38.17 ng/µl, with values ranging from 15.5 to 82.1 ng/µl (Figure 3b).

The Suffolk samples showed DNA concentrations between 14 and 73.9 ng/µl, with an average of 37.03 ng/µl (Figure 3c). The principle of spectophotometric method is based on UV absorption of biological substances: 260 nm corresponds to DNA/RNA, 280 nm to proteins, and 230 nm to other contaminants [7].

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

Sample ID	Abs260	Abs280	Abs230	260/280	260/230	concentration (ng/ul)	Sample Type
1MCN	0.695	0.385	0.46	1.81	1.51	34.7	dsDNA
2MCN	2.182	1.602	3.196	1.76	0.68	59.1	dsDNA
3MCN	0.08	0.042	0.069	1.9	1.16	14.2	dsDNA
4MCN	1.241	0.648	0.633	1.92	1.96	62	dsDNA
5MCN	0.495	0.256	0.388	1.93	1.28	24.7	dsDNA
1FCN	0.201	0.1	0.163	2.01	1.23	13	dsDNA
2FCN	0.209	0.12	0.306	1.74	0.68	10.4	dsDNA
3FCN	0.135	0.057	0.136	2.17	0.99	16.7	dsDNA
4FCN	0.094	0.057	0.125	1.65	0.75	12.6	dsDNA
5FCN	0.341	0.166	0.24	2.05	1.42	17	dsDNA
6FCN	0.039	0.002	0.047	1.95	0.83	19	dsDNA
7FCN	0.074	0.024	0.045	2.08	1.64	37	dsDNA
8FCN	1.735	1.124	1.825	1.54	0.95	86.7	dsDNA
9FCN	0.422	0.245	0.263	1.72	1.6	21.1	dsDNA
1MI	0.705	0.376	0.343	1.88	2.06	35.2	dsDNA

Sample ID	Abs260	Abs280	Abs230	260/280	260/230	concentration (ng/ul)	Sample Type
2MI	0.858	0.504	0.478	1.7	1.79	42.8	dsDNA
3MI	2.895	1.648	1.928	1.76	1.5	44.7	dsDNA
4MI	3.642	2.705	4.405	1.35	0.83	82.1	dsDNA
5MI	0.637	0.392	0.694	1.63	0.92	31.8	dsDNA
1FI	0.87	0.521	0.715	1.67	1.22	43.5	dsDNA
2FI	0.395	0.223	0.226	1.77	1.75	19.7	dsDNA
3FI	0.642	0.39	0.548	1.65	1.17	32	dsDNA
4FI	0.688	0.377	0.455	1.82	1.51	34.4	dsDNA
5FI	0.31	0.179	0.109	1.73	2.84	15.5	dsDNA
1MS	0.417	0.255	0.288	1.64	1.45	20.8	dsDNA
2MS	0.491	0.293	0.381	1.68	1.29	24.5	dsDNA
3MS	1.033	0.648	1.044	1.59	0.99	51.6	dsDNA
4MS	1.478	0.863	0.962	1.71	1.54	73.9	dsDNA
5MS	0.677	0.391	0.417	1.73	1.62	33.8	dsDNA
1FS	0.679	0.425	0.615	1.6	1.1	33.9	dsDNA
2FS	0.333	0.217	0.383	1.53	0.87	16.6	dsDNA
3FS	1.462	0.99	1.592	1.48	0.92	73.1	dsDNA
4FS	0.281	0.201	0.295	1.74	0.95	14	dsDNA
5FS	0.563	0.344	0.483	1.64	1.17	28.1	dsDNA

Note: Abs – Absorbance

DNA concentration values ranged from 10.4 to 86.7 ng/µl, with an average concentration of 34.7 ng/µl across all samples from the three breeds studied (Figure 1 and 2).

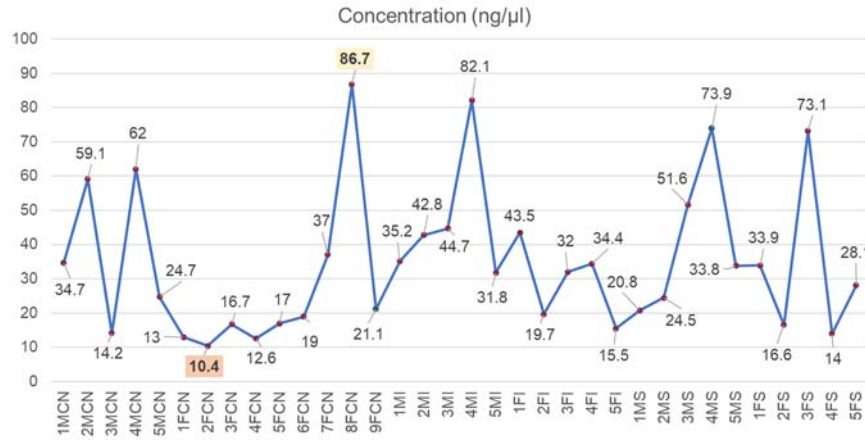


Fig 1. Extracted DNA concentration values, measured with Nanodrop ASP-3700 spectrophotometer (ng/µl

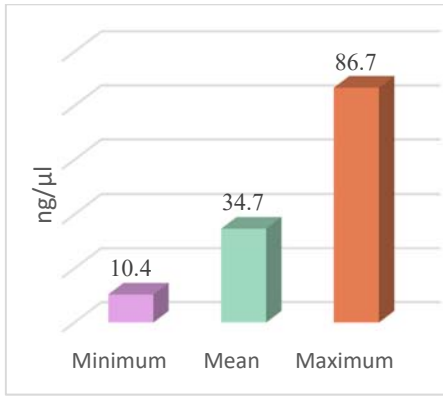
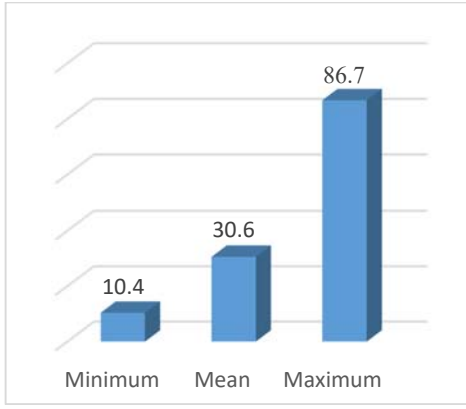
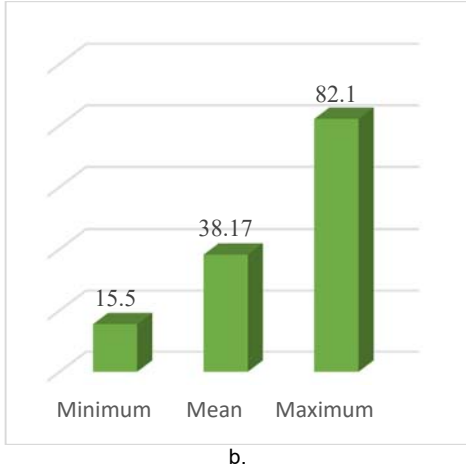


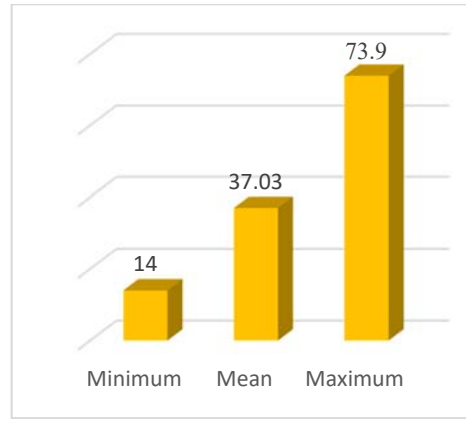
Fig. 2. Range and average DNA concentration across breeds



a.



b.



c.

Fig. 3. Extracted DNA concentration values (ng/µl) for: a. Teleorman Black Head, b. Île de France, c. Suffolk

DNA purity is typically indicated by an A260/A280 absorbance ratio within the range of 1.7-2.0. Ratios below 1.7 suggest protein contamination, while those above 2.0 may indicate the presence of other contaminants [8,9].

In our case, 17 samples had values within the range of 1.7 - 2, 13 had values below 2, and 4 samples had values above 2. (Figure.4). However, the values outside the normal range did not vary significantly, which will not affect the next stages of the work.

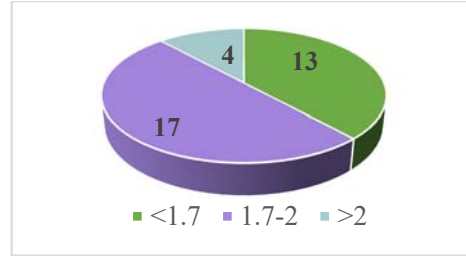


Fig. 4. Distribution of samples according to the value of the absorbent ratio A260/A280

In other studies [1] obtained an average DNA concentration of 79.258 ± 1.715 ng/µL and a purity of 1.66 ± 0.009 using a modified method of DNA extraction and isolation from nucleated cells in sheep blood.

Similarly, [10] reported an average DNA concentration of 27.7 ng/µL from bovine blood, with purity values showing A260/A280 ratios ranging from 0.68 to 2.13, using automated extraction with Maxwell™ 16 and 16 MDx instruments.

Also, in a study regarding DNA extraction from dairy products, conducted by [11], showed large variations in the quantity of DNA obtained using a comercial kit, 34-84 ng/µL.

These values obtained in this paper align closely with those in the literature and fall within standard purity ranges, confirming the effectiveness of the isolation method and ensuring reliability in subsequent analysis stages.

CONCLUSIONS

Following the isolation and quantification process, satisfactory values regarding the DNA concentrations of all 34 individuals from the three breeds were obtained, with these values being comparable to those reported by other authors.

Most of the isolated DNA samples fell within the optimal purity range of 1.7-2.0, based on the A260/A280 ratio, while the remaining samples were close to this range.

This step of isolating and quantifying DNA from sheep blood is crucial for proceeding with genetic analyses aimed at identifying SNPs in the myostatin gene across the three studied sheep breeds.

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