SCIENTIFIC HORIZONS

Journal homepage: https://sciencehorizon.com.ua
Scientific Horizons, 27(2), 31-42

UDC 577:636
DOI: 10.48077/scihor2.2024.31

Spectrophotometric method for determining the quantity and quality of DNA in animal breeding

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Article's History:
Received: 20.09.2023
Revised: 26.12.2023
Accepted: 24.01.2024

Abstract. In animal breeding, genetic methods have become the basis of breeding work and veterinary diagnostics. Therefore, their development and improvement is an actual direction of modern science. The aim of the presented work was to study the concentration and quality of nucleic acids obtained from venous blood of cattle for further genetic studies. For this purpose, a modified method of phenol-chloroform extraction, adapted for DNA extraction from blood, with subsequent

Suggested Citation:
Antane, V., Sarybayev, Y., Osserbay, A., Shatmanov, K., & Baltakhozhayev, T. (2024). Spectrophotometric method for determining the quantity and quality of DNA in animal breeding. *Scientific Horizons*, 27(2), 31-42. doi: 10.48077/scihor2.2024.31.

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spectrometric determination of DNA concentration and assessment of its quality were applied. As a result of this research, it was found that the average concentration of genetic material isolated from animal blood was 146.5±14.98 ng/µl. The main part of samples – more than 93% contained concentration of nucleic acids in the range from 50 to 200 ng/µl. At the same time, the time interval between DNA extraction and its spectrometric determination of concentration and quality of genetic material by the ratio of optical density at A260/A280 wavelengths during a year did not have significant changes on its parameters. The used method of nucleic acid extraction in 94% allowed obtaining samples of good quality suitable for further genetic studies. A correlation of 43% (P<0.001) was obtained between the concentration of genetic material and its quality. The coefficients of repetition of intra-laboratory studies of the results of extraction and spectrometric analysis were at the level of 97% (P<0.001), which indicates that this method of obtaining nucleic acids is adapted for its use in animal husbandry. The use of this method of DNA extraction allows obtaining quality material from animals with minimal economic costs for its further use in genetic research

Keywords: genetic material; nucleic acids; optical density of the solution; correlation; repeatability; nucleic acid extraction

INTRODUCTION

Genetic methods have recently gained popularity in animal breeding. Being the most informative and accurate diagnostic methods in determining infectious diseases, they are often used in veterinary practice, as well as in breeding programmes using genomic selection. But for this purpose, only high-quality nucleic acid samples with known concentration are needed, which allows high accuracy of the research. For most medical laboratories, the work of quantifying and characterizing genetic material has already become routine. While in animal breeding, this direction is just developing and therefore needs both research of theoretical bases and practical work on adaptation of this method to physiological parameters of different types of agricultural and domestic animals.

In Kazakhstan, such studies are conducted mainly only at the level of nucleic acid extraction from animals of certain species and determination of concentration and quality of the obtained DNA. Since these parameters determine the further use of such samples for genotyping (Kulibaba *et al.*, 2023). Thus, A. Ibadullayeva *et al.* (2022) conducted experiments to study different methods for the isolation of genetic material from sheep of Kazakhstani Edilbai breed from tissue preparations (pieces of auricle). The use of classical and commercial methods for DNA extraction showed that the salt method allowed obtaining the most suitable material for further studies.

D.I. Kabylbekova *et al.* (2023) conducted similar studies. They analysed the quality and concentration of nucleic acids suitable for genetic studies of the equine population in Kazakhstan. Commercial kits were used in these studies, which proved to be effective in isolating genetic material from this animal species. Similar studies were carried out by V.A. Ulyanov *et al.* (2023). The object of the study was a wild saiga species. In this work, only commercial kits were used for the isolation of nucleic acids from skin and its derivatives, with subsequent assessment of efficiency by electrophoresis. Similar work has also been carried out by K.B. Adyrbekova and K.B. Isbekov (2022) on isolation of DNA-material of fish for subsequent genetic study. In this work, a comparative analysis of DNA obtained by two methods adapted for aquaculture was also carried out.

Considering that in the Republic of Kazakhstan there is quite a large population of cattle, accordingly, and with this kind of animals were carried out similar work. Sh.D. Orkara *et al.* (2022) investigated the effect of two commercial foreign kits for DNA extraction from cow hair follicles and the phenol-chloroform method for subsequent genotyping by nucleotide polymorphisms markers (SNPs). As a result of this study, the authors concluded that both methods provided genetic material suitable for further work. Unlike the other method, the use of the PureLink™ Genomic DNA Mini Kit provided high quality nucleic acids, which contributed to a more accurate result. The only disadvantage of this study was the limited number of animals involved in the study – only 8 animals. Thus, experiments on isolation of genetic material from cattle in Kazakhstan are rather limited and need further research. This is facilitated by significant selection work in cattle breeding, aimed at studying and evaluating productive qualities of dairy animals to determine breeding value.

In this work, more in-depth studies were conducted on the influence of several conditions in the isolation of DNA from blood cells from cows, covering a significant population and considering laboratory errors. In this connection, the aim of the work was the spectrophotometric determination of qualitative and quantitative characteristics of genetic material isolated from the blood of cattle using the phenol-chloroform method.

LITERATURE REVIEW

Nucleic acid extraction is an important component of genetic analysis. According to M.N. Emaus *et al.* (2020), insufficient purification of nucleic acids during their

extraction inhibits amplification of genetic material and reduces the sensitivity of the method. Therefore, numerous methods have been developed to simultaneously obtain and purify genetic material. The most commonly used chemical methods for extraction of nucleic acids from tissues in laboratories are the centrifugation in caesium chloride gradient with ethidium bromide (Nasir *et al.*, 2017); phenol-chloroform extraction and its modifications (Piskata *et al.*, 2019; Roy *et al.*, 2020); cetriltrimethylammonium bromide (CTAB) extraction (Kouakou *et al.*, 2022), chelate extraction using Chelex resin (Simon *et al.*, 2020; Gautam, 2022); alkaline extraction (Dairawan & Shetty, 2020), and one of the most recent developments is the purification method using cellulose chromatography (Baiersdörfer *et al.*, 2019). Each of the above methods has advantages and disadvantages.

According to the information of A. Ibadullayeva *et al.* (2022), the advantage of chemical methods is the relatively inexpensive price of reagents, but their significant toxicity and low efficiency compared to commercial kits gradually reduces their use. To reduce the negative impact of reagents and to improve the quality of extracted genetic material, some authors carry out several modifications. Thus, K.P. Bojang *et al.* (2021) indicated that of the four modifications of the CTAB protocol, the simplest method was the most effective. Not the last factor in using different methods is the labour intensity of their application.

Almost all the above methods are quite labour-intensive and unsafe for the health of the personnel who perform them. Therefore, recently, most laboratories and genetic companies use ready-made commercial kits to obtain and purify native nucleic acids (Yevstafieva *et al.*, 2019). But even commercial kits differ in the amount of DNA isolated from the sample and its quality. Thus, in the work of C. Butler *et al.* (2022), authors extracted nucleic acids from milk using four kits, followed by spectrophotometric control. This work confirmed differences in DNA amounts between kits. The Norgen Biotek test kit provided significantly higher DNA concentration measured at ratios of 260/280 and 260/230. Therefore, it is better to use kits from reputable genetic companies to obtain a higher result. Thus, in the work of S. Tsuji *et al.* (2019) the DNeasy Blood and Tissue DNA extraction reagent kits DNeasy Blood and Tissue DNA extraction Kit and PowerWater DNA Extraction Kit from the German company Qiagen were the most efficient for animal DNA extraction.

In addition, commercial kits contain the most novel and effective approaches that allow obtaining the highest quality samples of genetic material (Shahsavari *et al.*, 2022). Thus, the use of magnetic beads in modern kits, according to P. Oberacker *et al.* (2019), provide high performance in obtaining plasmid, genomic and total DNA and RNA regardless of the origin of the material. In a study by V. Ooi *et al.* (2023), the authors produced amplification results from low cellular material comparable to DNA extraction from skin using a novel eluent "High Volume-Cetyltrimethyl Ammonium Bromide-Phenol-Chloroform-Isoamyl Alcohol" (HV-CTAB-PCI).

Given the considerable variability between methods in the isolation, sequencing and bioinformatic approaches to assessing genetic material, K. Greathouse *et al.* (2019) found that it was the extraction methods that made the most noticeable differences. This influenced the work to investigate phenol-chloroform extraction of DNA from ruminant blood and to determine the laboratory bias in the studies.

MATERIALS AND METHODS

The experimental part of the work was carried out in 2021-2022. For this purpose, laboratory determination of quality and concentration of DNA isolated by phenol-chloroform extraction method from 137 samples from cattle blood was performed. Blood samples for the study were taken from productive dairy cows in "Baiserke-Agro" LLP, Talgar district, Almaty region, Republic of Kazakhstan. Blood was collected from the tail vein into vacuum tubes "Acti-Fine®" with anticoagulant. Ethylenediamine tetraacetic acid (EDTA), which is recommended in protocols of work with nucleic acids as a specific anticoagulant to reduce the error in the quantitative determination of DNA and RNA, was used as a blood clotting agent.

The extraction of genetic material was carried out using the phenol-chloroform extraction method with slight modifications. For this purpose, 300 µl of lysing buffer based on Tris-HCl and proteinase K from Quaigen Company were added to a microtube with 100 µl of whole blood. Subsequently, it was thermostated for 3 hours at 56-58°C. A Thermal Mixer from Thermo Scientific (USA) was used for this purpose. After incubation, 400 µl of phenol-chloroform-isoamyl alcohol mixture in the ratio of 1:1:1 was added to the solution and stirred. After 15-20 minutes, centrifugation was carried out at 6000 rpm for 10 minutes using mySPIN 12 centrifuge from Thermo Scientific (USA). Using a micropipette, the supernatant was collected and transferred to a new tube. An equal volume of phenol-chloroform mixture with isoamyl alcohol was added, and the above procedure was repeated 3-4 times.

Cold (-20°C) 96% ethanol alcohol was added to the obtained supernatant and the tube was left at -20°C for 20 minutes in the freezer, partial precipitation of DNA was observed. To accelerate this process, centrifugation at 6000 rpm for 10 minutes was performed. The supernatant was removed using a micropipette and the precipitate was washed several times with 400-500 µl of 70% ethyl alcohol solution. After completion of washing, ethanol was removed as much as possible using a micropipette, and its residue in the tube was allowed to volatilize by thermostating with the lid open at 37°C.

The resulting genetic material was further diluted using DNA dilution buffer from Evrogen.

DNA quality and concentration were checked and evaluated in the laboratory of Green Biotechnology and Cell Engineering of the Kazakhstan-Japan Innovation Centre at the Kazakh National Agrarian University. For this purpose, spectrophotometric method of DNA concentration determination by the level of absorption of certain wavelengths using Nanodrop 2000 spectrophotometer according to the instrument operation manual was used (Thermo Fisher Scientific, 2009). Qualitative and quantitative evaluation of DNA isolated was carried out in a 2 µl sample at 20°C at 230, 260 and 280 nm. The studies were carried out on the next day after the isolation of genetic material, two months later and one year later. The results of measurements from the instrument database were transferred to a spreadsheet format for further statistical processing in Tibco Statistica 14.0.1 software. For this purpose, using the methods of mathematical-statistical analysis and descriptive statistics, the influence of the optical density of samples with the concentration and quality of nucleic acids for further generalization, preparation of conclusions and recommendations for subsequent works were calculated.

RESULTS

For the study, 155 venous blood samples were collected from clinically healthy, productive dairy cows. As a result of extraction, nucleic acids were obtained from only 137 samples. Part of samples, through blood coagulation, which occurred during transport and storage of samples did not allow extraction of genetic material from them, so they were excluded from further work. As a result of this research, an average of 146.5±14.98 ng/µl of total nucleic acid concentration (DNA and RNA combined) was extracted from cattle blood. The limits ranged from 31.5 to 1114.4 ng/µl (Fig. 1).

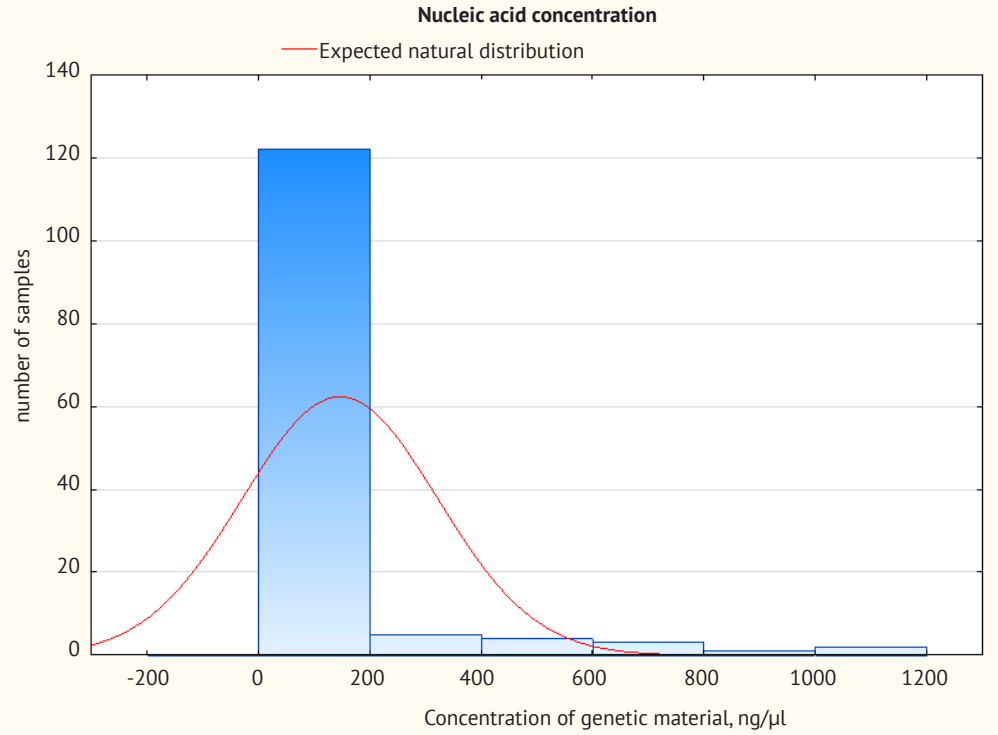

Figure 1. *Graph of distribution of blood samples depending on the isolated concentration of nucleic acids*
Source: *compiled by the authors*

The obtained graph, considering the grouping of sample results by DNA concentration, corresponds to a normal distribution. Considering that nucleic acids in blood cells are contained only in leukocytes, and their content in blood varies widely among animals even within a species, it is worth expecting certain fluctuations in DNA concentration in samples, which should correlate with the amount of this fraction of formational elements in controlled animals. In addition, the question of the maximum possible DNA yield and DNA loss during isolation by different methods remains open. Most samples had nucleic acid concentrations between 50 and 250 ng/µl. When the initially non-natural results were discarded and only averaged values within three sigma were left, this graph allowed the determination of the modal class and associated classes indicating the nucleic acid content of bovine blood (Fig. 2).

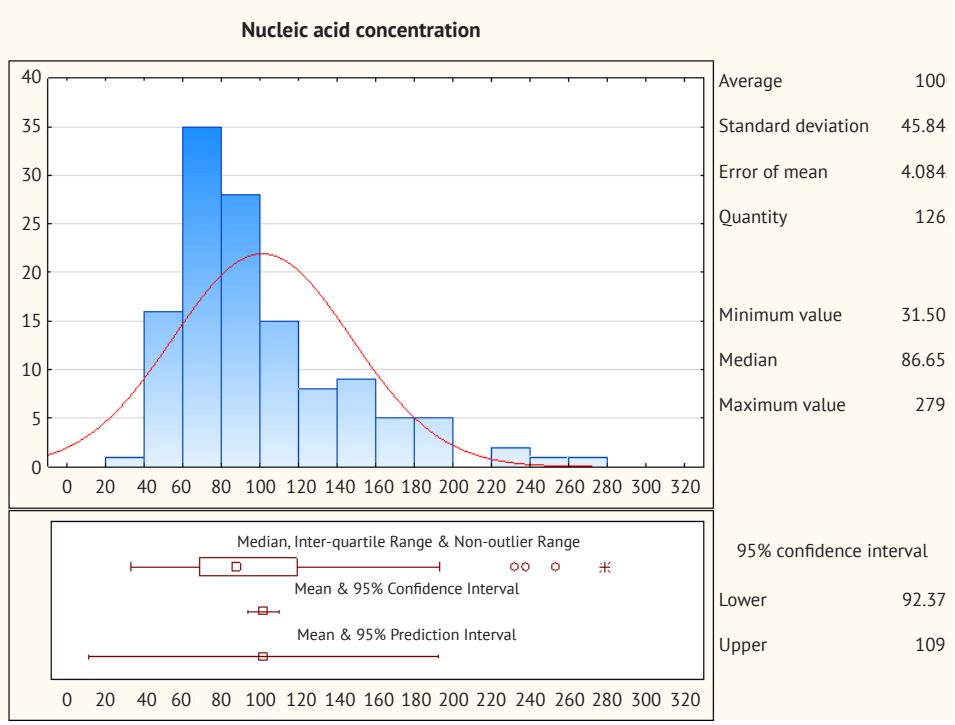

Figure 2. *Graph of the distribution of the number of samples depending on the concentration of nucleic acids*
Source: *compiled by the authors*

The modal class was samples with a concentration of genetic material between 50 and 100 ng/µl, which corresponded to 59.5% of the results of all studies. Furthermore, samples with concentrations of 100 to 150 ng/µl and 150 to 200 ng/µl were classified as high percentage classes, which accounted for 20.6% and 12.7%, respectively. These three classes totalled about 93%. Therefore, it can be stated that for clinically healthy cattle, the value of natural nucleic acid concentration will be between 50 and 200 ng/µl. Measurement of the optical density of solutions with extracted nucleic acids was carried out in several steps. The first study was carried out the day after the extraction of genetic material, for this purpose, only a minor part of samples was analysed, all the rest were stored in a freezer at -20°C. Most samples (n=82) were analysed two months after extraction from biological material. This was due to the recommendations of some researchers to keep the solution with nucleic acids after extraction for some time. And the third stage of the analysis was already connected with determining how long storage affects the preservation of genetic material. The graphical results of this study are presented in Figure 3.

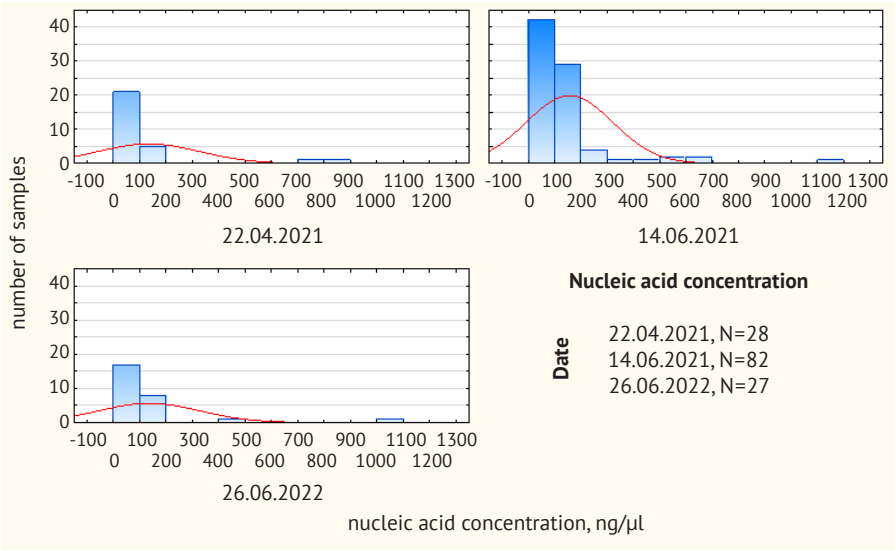

Figure 3. *Concentration of nucleic acids in samples depending on storage time after extraction*
Source: *compiled by the authors*

As follows from the presented graphical material, regardless of storage time, the profile of nucleic acid content remained practically the same and corresponded to a normal distribution. It is noteworthy that in all studies, the bulk of samples contained the concentration of genetic material at the level of 120-150 ng/µl (Table 1).

Table 1. *Results of determining DNA concentration depending on sample storage time*

Date of the study	N, samples	M±m, ng/µl	Min, ng/µl	Max, ng/µl
22.04.21	28	133.6±36.78	31.5	830
14.06.21	82	154.8±18.15	48	1114.4
26.06.22	27	134.8±36.92	44.3	1019.1

Note: *Min – minimum value; Max – maximum value*
Source: *compiled by the authors*

As a result of the work, there was a slight increase in DNA concentration two months after its isolation, but this could be the result of a simple increase in the number of measurements (more than 3 times) due to the averaging of results. But despite this increase in DNA values, the difference between the groups was not significant (P>0.1). Nevertheless, this nuance needs to be further investigated and tested on a larger sample. The conducted studies indicate, during the year, there was almost no change in the concentration of nucleic acids during its storage. This insignificant difference between the results of their measurement in samples allows using extracted DNA in this interval, without loss of informativeness, for genetic research in animal breeding.

To check the quality of the genetic material extracted from blood, already proven methods that are based on the ratio of the optical density of the solution at wavelengths of 260, 280 and 230 nm were used. This approach is related to the properties of nucleic acid molecules to absorb and reflect waves of different intensities. The most recommended method is the difference of indices at wavelengths 260 and 280. The A260/A280 ratio for pure nucleic acids is in the range of 1.8-2. The absorption optimums are about 1.8 for DNA and about 2 for RNA. Values less than 1.8 indicate incomplete purification of the preparation and residual concentrations of protein molecules. The presence of samples with a ratio higher than 2 indicates inadequacy of the reagents used or excessive physical strain, which causes DNA degradation and, as a consequence, the presence of free nucleotides in the solution. Since in the research the determination of not pure fractions of nucleic acids, but their mixture was carried out, the optimal difference was set between 1.7 and 1.9. All samples with the value of the absorbance difference falling within this range were considered suitable for further genetic study. All other values were grouped in the ratio less than 1.7 and greater than 1.9 (Fig. 4).

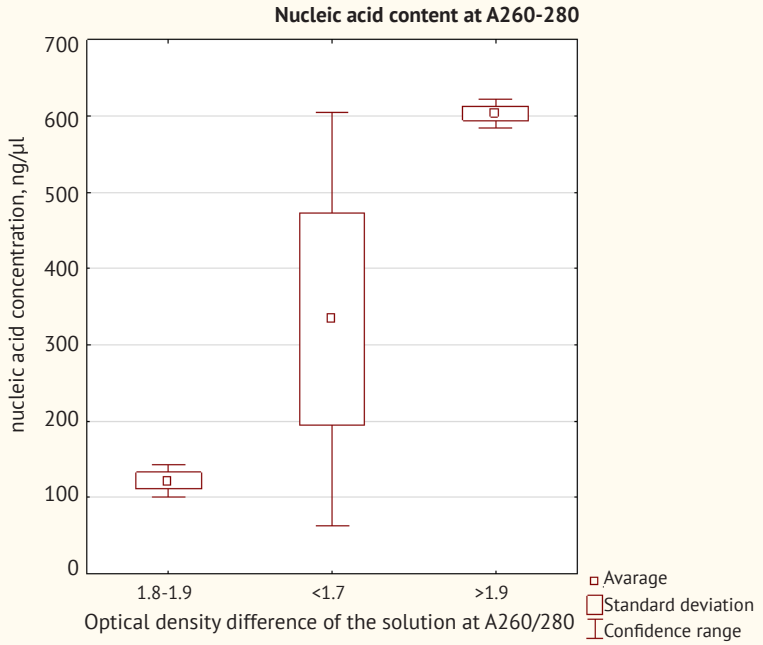

Figure 4. *Graphic display of the distribution of nucleic acid samples depending on the difference in optical density at wavelength A260/A280*

Source: *compiled by the authors*

As can be seen from the presented graphs, the amount of nucleic acids varied greatly in samples with a density difference of less than 1.7 A260/A280, which may have been a marker of impaired extraction of genetic material. Whereas in other groups no such variation was observed (Table 2).

Table 2. *Indicators of the concentration of nucleic acids in samples with different optical densities at wavelength A260/A280*

Optical density at A260/A280	n, samples, (%)	M±m, ng/µl	Standard deviation	Minimum value, ng/µl	Maximum value, ng/µl
Less than 1.7	9 (7%)	334.1±138.5	415.5	31.5	1114.4
1.7-1.9	125 (91%)	122.1±10.7	119.8	44.3	412.3
More than 1.9	3 (2%)	603.8±9.6	16.7	588.8	621.8

Source: *compiled by the authors*

At values of optical density A260/A280 above 1.7, a direct correlation between optical density and DNA concentration in the sample was clearly observed, with the correlation coefficient between these parameters being 0.43 (P<0.001). Whereas in samples with optical density lower than 1.7, the variation of the concentration of genetic material ranged from 31 to 1114 ng/µl, and these were the borderline values from the array of all studies conducted. Therefore, samples with optical density below 1.7 are not recommended for use in further genetic studies.

Based on the rather high statistical informativeness of the A260/A280 ratio indicator in the evaluation system of extracted genetic material, analysis of variance was performed to clarify the strength of the effect of the amount of nucleic acids in solution on its optical difference at A260/A280 wavelengths. To obtain a more reliable value, samples that, initially, were considered not logical were also excluded. It is worth noting that this group did not include all samples with an optical difference below 1.7. As a result of the single-factor analysis of variance, a reliable influence of the factor of nucleic acid concentration and quality on the indicator of its optical density was found, with an influence power of 24.4% (P<0.001; F=21.6). Another indicator used in the analysed literature to characterize the quality of nucleic acids is also the ratio of the optical density of the solution at A260/A230 wavelengths. In this study, the variation of this ratio was between 0.65 and 1.83, while the average ratio was 1.2±0.02. This result was significantly different from that reported in the literature, where the value of this ratio is between 1.8 and 2.2. The analysis of this situation may be related to the use of a new DNA dilution buffer from Evrogen. Therefore, it is planned to compare the difference of solution optical density at A260/A230 using different buffers for nucleic acid dilution at the last extraction step in the next studies.

One more area of work was to investigate the repeatability of nucleic acid concentration results when extracting nucleic acids from the same sample. This was made possible through coagulation of blood in several tubes during transport of samples to the laboratory. Therefore, this portion of the samples was replaced with samples from tubes where there was sufficient biological material to duplicate the extraction process of genetic material. This approach resulted in the extraction of nucleic acids from 5 samples. The extraction of genetic material was carried out in parallel at the same time to avoid errors in the procedure. The results of such work are presented in Table 3.

Table 3. *Results of parallel extraction of nucleic acids from samples of biological material*

Sample number	Ind. animal number	First sample		Second sample	
		Concentration of n.a.	A260/A280	Concentration of n.a.	A260/A280
83	147	50.8	1.79	54.3	1.78
60	723	59.1	1.76	62.2	1.82
107	758	127.2	1.85	80.7	1.83
66	856	125	1.84	72.3	1.83
81	498	83.4	1.79	152.1	1.81

Note: *n.a. – nucleic acids*
Source: *compiled by the authors*

The presented tabular material indicates that there was no significant difference between the nucleic acid concentration indices. At the same time, the genetic material, according to the results of optical density difference at A260/A280 wavelengths, was of very high quality in both variants. Therefore, the repeatability of results between parallel studies was determined by correlation analysis. As a result, a correlation coefficient of 0.97 (P<0.001) was obtained (Fig. 5).

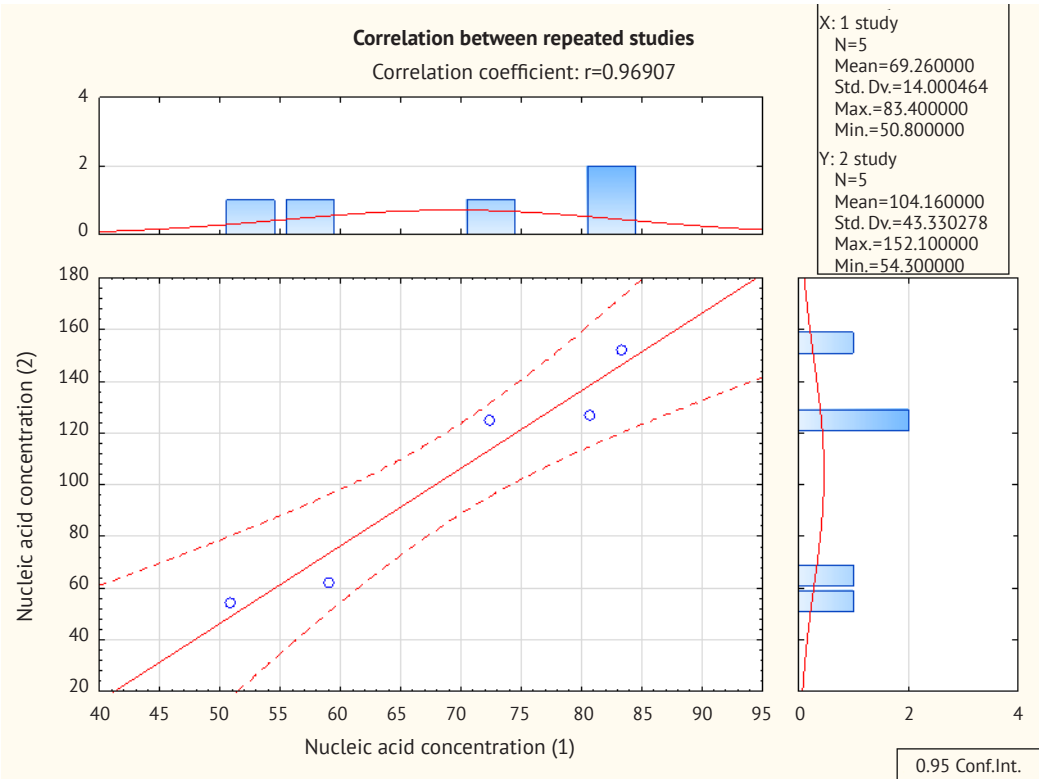

Figure 5. *Results of correlation analysis of repeated studies of nucleic acid extraction from blood samples*
Source: *compiled by the authors*

The above studies indicate that the method of phenol-chloroform extraction of nucleic acids can be successfully used to work with biological material from animals. The obtained material has the necessary concentration and quality for subsequent genetic studies.

DISCUSSION

Modern livestock breeding increasingly uses genetic methods in breeding work and veterinary diagnostics. And, in this regard, the isolation of genetic material is a priority task in genetic research. Venous blood was chosen as an object for isolation of nucleic acids. This was due to the versatility of this body system for carrying out diagnostics of the animal's condition. It is used for clinical diagnostics of the physiological state of the organism, immunological testing and other examinations. Any tissues of the organism containing cells are suitable for DNA isolation. In most of the analysed studies, skin derivatives (hair follicles, skin biopsy pieces) or tissue pieces left after breeding manipulations (ear plucking) were used to isolate genetic material. The use of blood is associated with a more labour-intensive process of its collection, but considering that such manipulations are carried out at least twice a year (according to veterinary and sanitary rules of research of productive cows, whose products are used for food preparation) such material for genetic research becomes the most universal object in animal breeding, well, or in dairy cattle breeding at least (Króliczewska *et al.*, 2023).

The phenol-chloroform method for nucleic acid extraction was used in the research because it is reported by researchers such as S.A. Sakyi *et al.* (2023) to be much cheaper than other commercial methods. And in addition, it allows maximizing the yield of DNA from the sample. This is especially relevant given the relatively low concentration of leukocytes in the blood, almost the only mature cells with nuclei, which are the carriers of DNA. When nucleic acid extraction was performed from cow blood, an average of 146.5±14.98 ng/µl DNA per sample was isolated. This result was significantly higher than in the study of M. Akinwole and A. Babarinde (2019), A. Ibadullayeva *et al.* (2022), in which the amount of genetic material was only 43.4-48.5 ng/µl when DNA was extracted from ear pluckings by the same method. This difference is quite significant and can be related only to the number of cells that contain nucleic acids. Since in the ear pluck a significant part of the tissue is represented by cartilaginous base, in which DNA is practically absent, the concentration of genetic material was reduced. This could be the explanation for the different concentration of genetic material extracted using the same method. Moreover, that when nucleic acids were extracted from ear pluck by other methods, the concentration of DNA was found to be about the same level as with the phenol-chloroform method, namely 50-61.2 ng/µl.

But in the studies of J. Carvajal-Agudelo *et al.* (2021) on the extraction of nucleic acids from blood of wild an-

imal species using the phenol-chloroform method and other commercial kits, lower levels of nucleic acids up to 25 ng/µl were also obtained. In these studies, dried blood samples from dogs were used for analysis, which could significantly affect the number of cells with nuclei. By drying the blood, some leukocytes may have broken down, and this could have affected the isolated DNA concentration in the dried blood (Mazur *et al.*, 2022). This assumption was partially confirmed in the study of H. Mayta *et al.* (2019) who performed DNA extraction from whole blood and clotted blood samples. As a result of such work, a greater concentration of genetic material was extracted from blood clotted blood as the concentration of cells passed in them, but the cost of extraction also increased significantly. It follows from this that, regardless of the state of blood tissues, it can be used for isolation of genetic material, but the highest yield of nucleic acids with the lowest financial costs can be obtained from whole or stabilized blood (Irgashev *et al.*, 2020).

Very different results for DNA extraction from animal blood were obtained in the work of L. Le Clercq *et al.* (2023). In their study, using a commercial Extract-Now™ Blood DNA Mini Kit, the concentration of nucleic acids from blood was obtained at the level of 4 ng/uL, which corresponded to 4000 ng/µl, which is an order of magnitude higher than the results obtained using the phenol-chloroform method. Therefore, such differences in the concentration of genetic material obtained from the studies of the above authors and those obtained in this article remain unresolved and need to be investigated more thoroughly. In particular, this will be facilitated by studying the influence of the leucocyte content in blood on the concentration of nucleic acids during its extraction. This direction will be a priority in future works.

But no less important factor than the quantity of genetic material obtained from a biological sample is its quality. In the conducted studies, the only factor that could testify to the quality of the obtained nucleic acids was the difference in the optical density of the solution at wavelengths 260, 280 and 230 nm. As a result of the analysis, the quality control of the isolated DNA was carried out by the A260/A280 ratio. In this case, 93% of samples had quality parameters that meet the requirements for genetic testing. This result indicates that the method used for DNA extraction from such a biological object as blood is quite suitable. In similar studies conducted by Z. Piskata *et al.* (2019) indicate that when the phenol-chloroform method was used, the number of samples having the criterion as qualitative by A260/A280 ratio was also more than 90%. Whereas, when commercial nucleic acid extraction kits were used, this control method was not effective for several kits. Therefore, the optical density at A260/A230 was also used to control the quality of the extracted genetic material. This method, which is also used for spectrometric quality control in the conducted studies, was at the level of 1.2±0.02, while the norm is 1.8-2 (as an indicator of solution purity without protein molecules admixture). In the works, where the method of phenol-chloroform extraction was also used by G. Koetsier and E. Cantor (2019), A. Sophian (2021) the optical density ratio at A260/A230 was also below the recommended level of 1.24 and 0.81-0.87, respectively. Therefore, it is worth revisiting the compliance rates for the quality of extracted DNA in spectrometric analysis.

The laboratory reproducibility of the results is also an important factor. In this work, a correlation analysis method was applied between adjacent samples from the same biological sample. Such work resulted in a very high repeatability coefficient of 0.96 and statistically significant ($P<0.001$). Unfortunately, there were no similar works that studied the reproducibility of DNA extraction results from animal blood, but this assumption is confirmed in the work of A.J. Sepulveda *et al.* (2020), where very high repeatability rates of 91 and 92% were also obtained for DNA isolation from molluscs using a commercial Qiagen kit. But in contrast to such optimistic results, D. Burke *et al.* (2022) point out that when the same methods and test kits were used in two different laboratories, the results of quantitative isolation of genetic material differed significantly. Therefore, works on the unification of methods and their protocols are still quite relevant in genetic research not only in animal breeding, but also from the biological perspective in general.

In conclusion, it should be noted that the use of phenol-chloroform extraction of nucleic acids from animal blood is an effective, inexpensive, and reproducible method within the laboratory, which is reasonable to use in genetic studies in animal breeding.

CONCLUSIONS

The results of the application of phenol-chloroform method of extraction of genetic material from cattle blood and its further spectrometric analysis allow drawing the following conclusions and developing several directions for further works. The use of phenol-chloroform method of extraction of nucleic acids from such biological object as venous blood of cattle allowed isolating DNA in a concentration 146.5±14.98 ng/µl. At the same time, 93% of samples had concentration from 50 to 200 ng/µl.

Spectroscopic analysis with such time intervals as one day, two months and one year after extraction allowed stating that there were no significant deviations in the concentration of genetic material and its qualitative characteristics. No significant difference was observed between the groups. This indicates that the genetic material can be used for one year without loss of informativeness. The quality of the isolated genetic material determined by measuring the ratio of optical absorption density at A260/A280 wavelengths

indicates that more than 90% of the isolated samples were qualified as qualitative and suitable for genetic studies. The correlation between DNA concentration and quality was 43% (P<0.001). Intra-laboratory repeatability of the results of genetic material extraction and its spectroscopic study carried out from the same biological samples indicated a high correlation of 97% (P<0.001). Such repeatability of results indicates the efficiency of the method for its use in animal husbandry when working with blood.

The most promising directions for future work are to determine the influence of leukocyte concentration in blood sample on the results of nucleic acid extraction and to study the influence of different buffers for DNA dilution on optical density at wavelengths of 260 and 230 nm for further quality control of genetic material. These areas of research will increase the informativeness and reliability of the method in animal breeding.

ACKNOWLEDGEMENTS

None.

CONFLICT OF INTEREST

None.

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Спектрофотометричний метод для визначення кількості та якості ДНК у тваринництві

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Анотація. У тваринництві генетичні методи стали основою селекційної роботи та ветеринарної діагностики. Тому їх розвиток та удосконалення актуальний напрямок сучасної науки. Метою представленої роботи стало вивчення концентрації та якості нуклеїнових кислот отриманих з венозної крові великої рогатої худоби для подальших генетичних досліджень. Для цього було застосовано модифікований метод фенол-хлороформної екстракції, адаптований для виділення ДНК із крові, з подальшим спектрометричним визначенням концентрації ДНК та оцінкою її якості. У результаті проведених досліджень було встановлено, що середня концентрація генетичного матеріалу, виділеного з крові тварин, становила 146.5±14.98 нг/мкл. Основна частина зразків – понад 93 % містила концентрацію нуклеїнових кислот в інтервалі від 50 до 200 нг/мкл. При цьому часовий інтервал між екстракцією ДНК та її спектрометричним визначенням концентрації та якості генетичного матеріалу за співвідношенням оптичної густини за хвиль A260/A280 упродовж року не спричиняв суттєвих змін на її показники. Використаний метод виділення нуклеїнових кислот у 94 % дав змогу одержати зразки доброї якості, придатні для подальших генетичних досліджень. Між концентрацією генетичного матеріалу та його якістю отримано кореляційний зв'язок на рівні 43 % (P<0,001). Коефіцієнти повторення внутрішньо лабораторних досліджень результатів екстракції та спектрометричного аналізу виявилися на рівні 97 % (P<0,001), що вказує на адаптованість цього методу отримання нуклеїнових кислот для його використання у тваринництві. Застосування цього способу екстракції ДНК дало змогу з мінімальними економічними витратами отримати якісний матеріал від тварин, придатний для його подальшого використання в генетичних дослідженнях

Ключові слова: генетичний матеріал; нуклеїнові кислоти; оптична густина розчину; кореляція; повторюваність; екстракція нуклеїнових кислот