Chicken nuclear DNA in chicken egg whites

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Article

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Abstract

Poultry eggs contain high-quality proteins, fat, vitamins, and minerals necessary for human nutrition as food and ingredients in the food industry. Egg whites have exceptional foaming, emulsifying, gelling, and heat setting properties, making them popular for use in baked goods. Intensive study of egg whites to date has now settled on a stable opinion that there are no cells, and no DNA, in them. However, this study demonstrates the possibility of chicken nuclear DNA extraction from outer thin whites of chicken eggs.

Introduction

Nowadays, we cannot imagine our life without poultry eggs, as they contain high-quality proteins, fat, vitamins, and minerals necessary for human nutrition (Zhu et al., 2018; Li-Chan and Kim, 2008). Main components of eggs are the shell (8-11%), albumen (or white) (56-63%), and yolk (27-32%) (Zhu et al., 2018; Kovacs-Nolan et al., 2005). Egg whites consist of water (87.9–89.4 %) and proteins (9.7–10.6%), with carbohydrates (0.4–0.9%), ash (0.5–0.6%), and trace lipids (Kovacs-Nolan et al., 2005). Among the proteins found mainly ovalbumin (54%–66%), ovotransferrin (12%–13%), ovomucoid (9.5%–11%), lysozyme (2.3%–4.5%) and ovomucin (1.5%–3.5%) (Zhu et al., 2018). Other proteins (total 153 proteins) found in egg whites with the development of proteomics (D'Ambrosio et al., 2008).

Egg whites have capabilities of foaming, emulsification, gelation and heat setting, and this made them an indispensable ingredient in the baking bakery products (Zhu et al., 2018). These properties are being studied (Nasabi et al., 2017; Babaei et al., 2019), and there is currently increasing attention to egg white application in developing biomaterials, especially medical biomaterials (Dong and Zhang, 2021). However, despite such interest, there is no mention of attempts to study the DNA content in egg whites. Eggshells had also been thought of until the isolation of DNA from them had been demonstrated (Strausberger and Ashley, 2001; Rikimaru and Takahashi, 2009). This fact led to the idea that egg whites may also contain DNA.

To clarify this assumption, let’s briefly discuss the main stages of egg formation. Thus, the egg formation occurs in the oviduct after the oocyte ovulation (Okumura, 2017; Nishio et al., 2018). During the oocyte maturation, the avian egg yolk accumulates in the cytoplasm. Therefore, the yolk is the cytoplasm content, and because of this nuclear DNA should not present here. In addition, mitochondria (i.e., mitochondrial DNA) are also absent in the yolk, since mitochondria are embedded in the cytoplasmic matrix only around the germinal oocyte nucleus (Okumura, 2017; Bakst and Howarth, 1977; Perry et al., 1978).

After maturation and ovulation, the oocyte is captured by the infundibulum of the oviduct, where the fertilization occurs (Okumura, 2017; Nishio et al., 2018). After capture by the infundibulum, the oocyte or fertilized egg is further enveloped with the membrane, outer perivitelline layer, chalazae, albumen, perialbumen, shell membrane, and calcified shell, in this order as the egg moves through the parts of the
oviduct: the infundibulum, the magnum, the isthmus, the uterus, the vagina (Okumura, 2017; Nishio et al., 2018; Menkhorst and Selwood, 2008).

As it mentioned above, avian eggshells (without inner and outer membranes) contain cells and nuclear DNA, as nuclear DNA was isolated (Rikimaru and Takahashi, 2009). The eggshell accumulates in the uterus of the oviducts by the eggshell glands (Nishio et al., 2018). The accumulation of egg whites also occurs in the oviduct, but in its other department, in the magnum. Synthesizing of egg whites occurs in the tubular gland cells of the magnum (Nys and Guyot, 2011; Muramatsu et al., 1991). Therefore, egg whites should accumulate in the same way as eggshells, but in a different part of the oviducts. In this regard, the accumulation of DNA in egg whites is possible, as it happens in eggshells (Strausberger and Ashley, 2001; Rikimaru and Takahashi, 2009). Thus, it was hypothesized that egg whites must contain cells, and nuclear DNA could be isolated from egg whites. This hypothesis is confirmed in this study, which demonstrates the possibility of isolating nuclear DNA from chicken egg whites. At the same time, it was proved that chicken egg whites contain nuclei, which means that they must also contain cells.

**Materials And Methods**

Experiments in this study were carried out under guidelines for the ethical and humane use of animals for research, according to the “Guidelines for accommodation and care of laboratory animals. Rules for keeping and care of farm animals” (Interstate Council for Standardization, Metrology and Certification, GOST 34088-2017).

**2.1. Egg samples.**

Six eggs from each of three chicken breeds and one commercial line (total 24 eggs) were used in this study. Leghorn Partridge, Pushkin, and Rhode Island Red breeds are from the Genetic Collection of Rare and Ending Chicken Breeds (St. Petersburg, Russia). Eggs were collected for three days and stored at 4–8ºC. Eggs of commercial layers are of Lohmann LSL-Classic from JSC Okskaya Poultry Farm (Ryazan, Russia). These eggs were of a first category (55–64g), according to Russian GOST31654–2012.

**2.2. DNA extraction from egg whites.**

Eggs were sponged away with running water and then rinsed with distilled water, and dried at room temperature. Then eggs were disinfected with 70% ethanol, and dried again at room temperature before being cracked. Cracked eggs, whites and yolks, were placed in a Petri dish. Then 3 ml of the outer thin white (Figure 1) from each egg were taken with a serological pipette into a sterile separate tube. Thereafter, trypsin (Trypsin 1:250, Sigma, USA) dissolved to a concentration of 0.1 g/ml in Versene solution (PanEco, Russia) in an amount of 2 ml was added. Samples were vigorously stirred on a vortex and incubated at 37ºC for 24 hr. Next, samples were centrifuged at 9,146 g force (Digicen 21R, Spain) at
room temperature (22 to 25°C) for 20 min. Supernatants were removed, and then each residue was dissolved in 0.1 ml of phosphate buffered saline pH 7.2–7.6 (PBS) (Eco-Service, Russia). Further, Extract DNA Blood kit (Evrogen, Russia) was used according to the manufacturer. DNA concentration and purity was estimated with NanoDrop 8000 Spectrophotometer (Thermo Scientific). Statistical analyzes were carried out using GraphPad Software (http://www.graphpad.com).

2.3. DNA analyzes.

18S ribosomal DNA based PCR test for avian and mammalian DNA identification was used (Zyrianova and Zaripov, 2022). Amplification from primers of this test was performed on the BIOER thermocycler (Hangzhou Bioer Technology Co, Ltd., China) using Phusion Hot Start II High-Fidelity DNA-polymerase (Thermo Scientific). Amplification conditions were used as follows. First, an initial denaturation at 95°C for 3 min was used. Then 40 cycles were performed: denaturation at 95°C for 15 s, annealing at 58°C for 15 s, and elongation at 72°C for 10 s. PCR fragments were analyzed on 1.7% agarose gels using tris-acetate-EDTA electrophoresis buffer. The agarose gel was stained with ethidium bromide and analyzed by a UVITEK-Cambridge imaging system. 100+bp DNA Ladder (Evrogen, Russia) was used for DNA marker.

PCR fragments of 97 bp length were cloned using the CloneJET PCR Cloning Kit (Thermo Scientific). Clones for each breed were sequenced using Sanger’s sequencing method (Evrogen, Russia). Sequences were analyzed using Gene Runner (Version 6.5.52) and NCBI BLAST. GenBank sequences of Gallus gallus 18S rRNA were used for the comparison: MK279380.1, MK279379.1, MK279378.1, XR_003078042.1, MG967540.1, KT445934.2, XR_006936397.1, XR_006936393.1, XR_006931663.1, XR_005840274.1, XR_005840272.1, MT889761.1, MT808178.1, HQ873432.1, FM165414.1, DQ018752.1, AF173612.1, and D38360.1.

2.4. Nuclear staining.

Trypsin solution (0.1 g/ml in PBS) in amount of 2 ml was added to 3 ml of the outer thin white, vigorously stirred, incubated at 37°C for 24 hr, and centrifuged at 9,146 g force (Digicen 21R, Spain) at 20°C for 20 min. The supernatant was removed, and the residue was dissolved in 0.1 ml PBS with Hoechst 33342 and incubated at room temperature for 5–10 min. Hoechst 33342 was prepared according to the protocol of Thermo Scientific. Probes of 0.02 ml were placed on a microscope slide. The imagination was done with Nicon Eclipse Ti microscope using Nikon Dm400 Fluorescence Filter Cube for DAPI (EX 340-380, BA 435-485).

Results And Discussion
3.1. Egg white samples and DNA yields.

Four different layers can be distinguished in the egg white (Fig. 1): the outer thin white, the thick white (or outer thick white), the inner thin white (not shown on Fig. 1), and the chalazae (or the inner thick white). Their proportions are about 23.3%, 57.3%, 16.8%, and 2.7%, respectively, which may vary depending on breed and environment (Li-Chan and Kim, 2008). The viscosity of thin whites is much less than of thick ones, due to the lower content of the gel-like glycoprotein ovomucin (Li-Chan and Kim, 2008). In this regard, the decision was made to use outer thin whites, since it was expected that their lower viscosity would be more beneficial in the fight against proteins of egg whites, as well as being easier to collect.

In an attempt to isolate DNA from outer thin whites, the problem of protein degradation should be addressed first. For this task, the serine protease trypsin was used. The trypsin concentration was increased, until the viscosity of the samples was completely overcome visually, and a light precipitate was appeared at the bottom of the tube after centrifugation.

Eggs of three breeds and one commercial line were used in the experiment. Together, 24 samples were tested. The average values of the extracted DNA are given in the Table 1. An unpaired t-test showed statistically significant difference (p < 0.05) only for Leghorn Partridge and Lohmann LSL-Classic, as well as Leghorn Partridge and Pushkin breeds.

The purity of DNA samples in most cases was found to be low (Table 1), and their difference to each other is considered statistically insignificant (p > 0.05; unpaired t-test with 95% confidence interval). However, this purity was sufficient for PCR (Fig. 2). There could be some reasons for the low purity of isolated DNA from outer thin whites. Firstly,

<table>
<thead>
<tr>
<th>Breeds</th>
<th>Yield (μg /ml of outer thin whites)</th>
<th>Purity (A260/A280)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leghorn Partridge</td>
<td>0.6380 ± 0.0545</td>
<td>1.4040 ± 0.0232</td>
</tr>
<tr>
<td>Pushkin</td>
<td>0.4500 ± 0.0577</td>
<td>1.5540 ± 0.0786</td>
</tr>
<tr>
<td>Rhode Island Red</td>
<td>0.4700 ± 0.0577</td>
<td>1.4820 ± 0.0750</td>
</tr>
<tr>
<td>Lohmann LSL-Classic</td>
<td>0.3880 ± 0.0348</td>
<td>1.3920 ± 0.0904</td>
</tr>
</tbody>
</table>

nothing is known about the properties of the egg white content regarding its interaction with DNA. Furthermore, perhaps the method of protein degradation of egg whites without loss of DNA can be improved.
3.2. DNA analyzes.

Isolated DNA samples were checked for presence of avian DNA by the PCR test based on 18S rRNA gene (Zyrianova and Zaripov, 2022) (Fig. 2). It is necessary to prove that isolated samples of total DNA contain chicken DNA, since internal pathogens can be detected in contaminated eggs. For example, it can be bacteria of *Salmonella*, which are considered almost the major pathogenic agents of internal contamination of chicken eggs (Wales and Davies, 2011). It was proved that the specific PCR fragment used in this PCR test based on the 18S rRNA gene is sufficient to distinguish between avian and mammalian species (Zyrianova and Zaripov, 2022). It is known that the 16S rRNA is an analogue of the 18S rRNA in prokaryotes. The 16S rRNA gene had been successfully used for diagnosis of prokaryotes by PCR tests based on this gene (Wellinghausen et al., 2009), including the detection of *Salmonella* (Trkov and Avgustin, 2003). PCR tests based on the 18S rRNA gene cannot detect prokaryotes. Thus, a positive test result used in this study indicates the detection of avian DNA, while a negative result would indicate the absence of avian DNA and the possibility of the presence of other DNA, including *Salmonella’s* DNA. In addition, we do not assume that any DNA from other animals that can be detected using PCR tests based on the 18S rRNA gene (for example, cattle, sheep, etc.) can be inside chicken eggs. All this allows to conclude that the positive result of the PCR test used proves that the isolated DNA samples in this study are of chicken origin (Fig. 2). Although, some minor fragments can be seen in Figure 2 compared to control chicken and cattle samples, this experiment clearly confirms the presence of chicken nuclear DNA in isolated total DNA samples of the outer thin whites. Minor fragments could be the result of contamination of DNA samples.

Furthermore, specific PCR fragments of 97 bp were cloned and sequenced (GenBank accession no ON005571). The sequence analysis revealed 98.97% identity (with one replacement C₁₇ → T₁₇) with 18 sequences of *Gallus gallus* 18S rRNA from GenBank. All these data indicate that the isolated DNA samples contain chicken nuclear DNA.

3.3. Nuclear staining.

In addition, Hoechst 33342 staining of the outer thin white samples was performed to see if the samples contain cell nuclei. Hoechst 33342 is widely used for labeling DNA in cells and allows visualizing of cell nuclei (Chazotte, 2011). As a result, Hoechst 33342 stained nuclei were visualized in outer thin white samples (Fig. 3). Hoechst 33342 stained nuclei appeared singly in these samples. This corresponds to a low concentration of extracted DNA. Nuclei were found at different stages of the cell cycle: in the interphase and in the mitotic stage (Figure 3, a, b). Moreover, necrotic nuclei were found in the samples (Figure 3, c). The stages of the nuclei were determined according to Crowley et al. (2016).
The ability to detect nuclei in samples of outer thin whites of chicken eggs is indisputable proof that they contain nuclei (i.e., and cells). Nuclei in egg outer thin whites were found at different stages of the cell cycle (Fig. 3). Necrotic nuclei in samples of outer thin whites (Fig. 3, c) most likely appeared due to insufficiently careful selection of trypsin treatment of samples or insufficient freshness of eggs.

**Conclusion**

This study demonstrates the possibility to isolate the nuclear DNA from chicken egg whites. The presence of the nuclear DNA in extracted DNA samples was proved by the 18S rRNA gene PCR test. Moreover, Hoechst 33342 staining of outer thin white samples revealed the presence of nuclei in them. Therefore, this study proves that there are nuclei (i.e., and cells), and nuclear DNA in the chicken egg white content. For a long time, the presence of DNA in avian eggs was considered possible only in the germinal disc (Steiner et al., 2011). The ability to isolate chicken nuclear DNA from chicken egg whites provides new insights into the molecular composition of avian egg whites. This fact raises the question of the need for a new research on the effect of this new component on the functional properties of egg white: their foaming, emulsification, gelation and heat setting capabilities. Moreover, the scientific direction of using egg whites as a substrate in 3-D cell cultures is currently being intensively developed (Dong and Zhang, 2021; Balaji et al., 2020). The discovery of their nuclei (i.e., and cells) in egg whites calls into question the possibility of using egg whites in 3-D technologies, because the resulting 3-D cultures are already mixed culture with egg white nuclei and egg white cells.

The presence of nuclear DNA and cells in egg whites overturns the idea of the molecular status of egg whites in general: egg whites are not just warehouses of proteins, but, perhaps, a factory for their production.

**Declarations**

**Data Availability**

All data generated during this study are included in this article and deposited in GenBank: accession no ON005571.

**Declaration of competing interest**

No conflict of interest exits in the submission of this manuscript.

**Acknowledgments**

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References


**Figures**
Figure 1

A cracked chicken egg in a Petri dish.
Figure 2

The agarose gel (1.7%) electrophoregram of PCR fragments after 18S rRNA gene testing of different total DNA samples from outer thin whites of chicken eggs: 2 – Leghorn Partridge, 3 – Pushkin, 4 – Rhode Island Red, and 5 – Lohmann LSL-Classic egg whites; 6 – chicken leg and 7 – cow blood (Zyrianova and Zaripov, 2022); 1, 8 – DNA marker; 9 – PCR negative control.
Figure 3

Images of nuclei of outer thin whites, treated with trypsin and stained by Hoechst 33342: a – interphase nucleus, b – mitotic stage nucleus, c – necrotic nucleus.