Using the heart rate of a bird embryo to monitor its condition

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Abstract. The article deals with the problem of artificial incubation of eggs of bird species, whose incubation is carried out for the first time and for which there are no data on the optimal temperature regime. The question of the need for using the methods allowing individual monitoring of the embryo is raised. The data from the literature on non-invasive methods of biological control of incubation, allowing changing the heart rate of a bird embryo to control its condition, are presented. It was found that the heart rate of embryos decreases as they develop. A similar effect on the heart rate, regardless of age, provides a decrease in temperature and lack of oxygen. Motor activity of the embryo creates interference check of the heart rate. The results of testing the device for determining the heart rate of the embryo by the optical method are presented. The circle of factors influencing the accuracy of measurements is defined. The possibility of lifetime determination of the duration of hypoxia of the embryo of birds by monitoring the heart rate is experimentally confirmed. The possibility of using the method of heart rate control to detect the degree of hypoxia of the embryo in order to determine the exact time of human operational intervention in the process of hatching of rare bird species is discussed.

1. Introduction

Modern agriculture is a science-intensive process that often requires the search for new solutions and methods [1]. One of the most important components of the technological process of breeding both farm and wild birds is artificial incubation. Over the past decades, thanks to the achievements of science and technology, high-tech incubators have been developed and manufactured, allowing one to ensure the established regime with maximum accuracy and to obtain indicators comparable to the results of natural incubation. It should be noted that the process of artificial incubation includes not only the use of modern equipment and compliance with the recommended regime. One of the necessary components is the use of biological control of incubation.

Different quality of the incubation egg, abnormal weather phenomena, malfunctions of the equipment and power supply networks, the notorious “human factor” affect the development of the embryo and, as a result, can cause a decrease in the results of incubation. The use of biological control allows identifying and analyzing factors that have a negative impact on the development of the embryo.

At present the main methods of biological control of incubation are ovoscopy, control of changes in egg weight and evaluation of incubation results. In case of need of more detailed research selective
opening of eggs with carrying out morphological, biochemical, hormonal and other researches can be carried out. It should be borne in mind that these methods have significant disadvantages, because they do not allow one to assess the state of the embryo in real time regime or lead to its death.

There are also studies that allow a lifetime assessment of the embryo. These include radiography, evaluation of the heart rate by methods of acoustic, ballistic and photoplethysmography [2].

However, they are not widely used, because they require the use of expensive equipment and, as a rule, are available only in specialized laboratories.

Nevertheless, it is safe to say that the incubation of most classical breeds of poultry, as well as species of wild birds, is used in wild breeding (common pheasant (Phasianus Colchicus Linnaeus, 1758); gray partridge (Perdix Linnaeus, 1758). African ostrich (Struthio Camelus Linnaeus, 1758), etc.), for a qualified specialist and with the use of modern technology, does not pose significant difficulties allowing one to obtain output rates of 80-85% of the fertilized eggs.

It should be noted that in recent years, both in the poultry industry and in the breeding of wild birds, there is a tendency to the emergence of an increasing number of eggs, incubation of which using known parameters does not allow achieving high rates of output. The reason for this phenomenon in poultry farming was the emergence of modern highly productive crosses of lines and breeds of domestic chicken (Gallus Linnaeus, 1758). The directed selection and application of the results of research in the field of genetics have achieved stunning results. The rate of growth and weight of modern breeds of meat birds greatly exceeds these figures of classic rock. For comparison, chickens cross Ross308 to 32-day age reach live weight 1815 g, while broiler chicken sample 1957 and 101 days of cultivation had a lower weight [3].

The high growth rate of modern crosses affected the course of embryogenesis.

According to the presented data (table 1), the rates of metabolic processes of the embryo and the amount of endogenous heat released have changed [4].

| Incubation day | The amount of heat released in meat and egg chickens, W/1000 eggs |
|---------------|-------------------------------------------------------------|
|               | Ross 308 | Ross 508 | White Leghorn | Dutch blue |
| 17            | 151.2    | 160.2    | 133.2        | 130.0       |
| 18            | 156.6    | 149.4    | 130.2        | 137.0       |
| 19            | 164.4    | 160.8    | 127.2        | 124.0       |
| 20            | 252.0    | 239.4    | 130.8        | 169.0       |

Biological control of embryogenesis of highly productive crosses incubated under the temperature-humidity regime used for classical breeds revealed several negative changes in the visceral systems of the embryo and therefore general decrease in hatchability [5,6,7].

At present the need to develop individual modes of incubation of specific crosses has been established [8]. In the breeding of wild birds, specialists are also increasingly faced with the need to incubate eggs of species for which there is no data on the optimal regime. Reducing the number of bird species on the verge of extinction, forcing nurseries and zoos to implement breeding programs of these species in captivity [9]. Obtaining data on natural incubation presents significant difficulties [10].

The classical method of developing the incubation regime of eggs of a new species or breed is based on the use of the following formula: incubation is carried out according to the known regime for a similar species; optimization is carried out empirically, based on biological control data. As practice shows, this method, allowing as a result obtaining satisfactory parameters, requires a large number of experimental data, the production of which with the use of classical methods of biological control is associated with significant time costs, as well as the need for a sufficient number of prototypes. The
limiting factor in the application of this technique in poultry farming is the need to develop optimal modes of incubation of a new cross in the shortest possible time period, and in the breeding of rare species of birds – the lack of the required amount of the experimental material.

All the above mentioned requires the search for new approaches to biological control of embryogenesis, allowing one to monitor the state of the embryo in real time, without damage to the shell, in the conditions of the operating poultry farm or nursery.

One of the directions of the research to find a method to monitor the state of the embryo in real time is to study the possibility of obtaining data on the heart rate of the embryo without destroying the shell [11]. Modern advances in optoelectronics enable fixing the light-transmitting ability of the eggs due to the reduction of blood vessels caused by the heart contractions, thereby allowing measuring the heart rate [12].

Several studies have shown the obvious effect of external influences on the heart rate of the bird embryo [13,14].

Considering the fact that the numerical value of the pulse is one of the main biological markers used in medicine and veterinary medicine [15], as well as the dominant role of this indicator when monitoring the state of the human body. It is possible to assume the prospects of using the heart rate index of the bird embryo obtained by the optical method without shell destruction as an objective method of biological control of incubation in real time [16,17,18,19].

2. Materials and methods

The experiment was conducted by a working group of specialists of Voronezh State Agrarian University named after Emperor Peter the Great and Voronezh State University in laboratory conditions. The laboratory is located on the territory of the reserve "Galichya Gora" of Voronezh State University, the address is the Russian Federation, Lipetsk region, Zadonsky district, Donskoye village (52°36'7.34 "sh., 38°55'43.45" - east longitudinal, altitude - 135.m). The eggs were incubated in an automatic incubator "R-COM 50 PRO" at a temperature of 37.6 °C and humidity 66%. The experiment was conducted from the 13th to the 20th day of incubation for chickens and from the 16th to the 32nd day for falcons. Heart rate monitoring was carried out using a sensor consisting of three IR visible LEDs and a photodiode. The sensor is connected to the amplifier by a shielded cable. As an analog-to-digital converter, an external PLM 5-2 USB sound card was used, the digitized signal was transmitted to a personal computer. Processing of the received signal was carried out in Adobe Audition 1.5 by methods of spectral estimation of the frequency response of the signal based on fast Fourier transform (figure 1). After the experiment, the incubation of eggs was continued in the incubator. The chickens had no external deviations.

Figure 1. Recording of waveforms of the heart rate of the embryo of the saker falcon (Falco Cherrug Gray, 1834).
3. Results
In order to test the experimental model of the device, an experiment on the effect of cooling the egg on the heart rate of the embryo was carried out. The values presented in table 2 confirmed the results of the early experiments. At the same time, the presence of arrhythmia of heart contractions was revealed. The data presented in table 2 were obtained by counting the number of heartbeats in 10 seconds, since the heart rate calculation for the duration of the pause between two adjacent heartbeats was uninformative due to the significant variability of their duration.

Table 2. Dynamics of changes in the heart rate of a 17-day-old chicken embryo with a decrease in temperature in the incubator from +37.6°C to +25°C

| Cooling duration, min | Heart rate per minute |
|-----------------------|-----------------------|
| 0                     | 229.2                 |
| 1                     | 226.8                 |
| 2                     | 222.0                 |
| 3                     | 216.6                 |
| 4                     | 213.0                 |
| 5                     | 208.0                 |
| 6                     | 203.2                 |
| 10                    | 195.2                 |
| 20                    | 174.3                 |
| 30                    | 157.2                 |
| 40                    | 146.4                 |
| 50                    | 139.0                 |
| 60                    | 134.6                 |
| 70                    | 129.1                 |
| 80                    | 126.8                 |
| 90                    | 124.7                 |
| 100                   | 122.6                 |
| 110                   | 121.9                 |
| 120                   | 121.0                 |

During the experiment, significant changes in the light-transmitting properties of the egg associated with the movements of the embryo were revealed (highlighted by circle on figure 2). In this regard, the calculation of the heart rate was carried out in areas of oscillograms free from distortions caused by the movements of the embryo. The accuracy of measurements was limited by the resolution of the software and was 0.1 heart rate per minute. Study of age-related changes in the heart rate of saker falcon embryos (figure 3) started on the 16th day of incubation and continued until hatching chickens. As a result, there was a decrease in the heart rate with the age of the embryo.

Figure 2. Section of the waveforms of pulse of the embryo of the saker falcon
As testing of the control device and registering the heart rate of the birds embryo was carried out on the basis of the breeding of rare bird species, an additional experiment on the use of the device under test to address specific tasks arising during incubation of eggs of rare birds was carried out, when the number of incubated eggs is limited, and the incubation of each egg is provided individually. In this regard, there is a need to monitor the state of the embryo in the last hours of incubation and in the process of hatching.

It is known that the transition to pulmonary respiration occurs before the eggshell hatching, and at first the chicken breathes air from the air chamber. However, the volume of air in the chamber is limited, and, as the amount of oxygen inside the egg decreases, progressive hypoxia stimulates muscle contractions and consequently the movement of the embryo. Thanks to this mechanism, the chicken implements hatching. However, if the chicken is weak, the hatching does not occur, and the chicken dies from the lack of oxygen.

Unlike death in the embryonic stage, timely assistance provided at the proper moment, gives a chance for the weakened chickens to survive, which in case of physical assistance lack would have died without hatching. The operation of this kind is risky, and so the specialists in the breeding of prolonged absence of hatching, face a dilemma whether to open the egg before, risking to damage healthy chickens or wait with the risk that the bird will suffocate if it is weak.

One possible solution of the problem is to assess the state of the embryo by the heart rate. In that case, if it is possible to establish the characteristic dependence of the effect of oxygen starvation on the heart rate of the embryo, it will be possible to accurately determine the moment when the mechanical opening of the egg is the only option to save the life of the chicken.

In order to test the possibility of assessing the degree of hypoxia of the embryo through the non-invasive heart rate control, the chicken egg on the 15th day of incubation was covered with a transparent film excluding contact of the shell with the surrounding air. The egg was incubated at 37.6 °C and placed in a heart rate monitor for a period of no longer than 15 seconds for each measurement. The observed on the graph (Figure 4) the dynamics of changes in the heart rate of the embryo correlates with the data obtained in the study of hypoxia of chicken embryos by invasive methods.

**Figure 3.** Changes in the heart rate of the saker falcon embryo associated with age.
4. Conclusion

The experiments confirmed the efficiency of the experimental device for optical heart rate determination of the bird embryo. At the same time, the ability to record an analog signal allowed one to identify the features of processing the data.

Even though the use of analog data makes it necessary to count the number of heartbeats at a certain time interval, this method avoids errors caused by the movements of the embryo in the egg. Preliminary visual assessment of the waveform gives the opportunity to make a count in a location free from fluctuations in the intensity of transmitted light of the ability of the eggs caused by noise (vibration surface, which is the device of an external electromagnetic and optical interference, movement of the embryo).

The revealed cardiac arrhythmia requires further research in order to develop a technique for calculating the heart rate, the optimal duration of the time period to obtain representative data, determining the threshold values of arrhythmia that are not the pathology of the embryo.

The possibility of registration of the decrease in the heart rate of the embryo as a result of hypoxia, can be considered an optical method of registration of the heart rate as an available method of determining the time of surgical intervention in the process of hatching chickens. Considering that when breeding species of birds under threat of extinction, the value represents each chicken, and operative intervention in the process of hatching is a common practice to nurseries. The way allows one with high accuracy to determine the critical point of hypoxia and improve the hatchability.

In the course of experimental studies, the state of the bird embryo was monitored by a non-invasive method. Analysis of the data indicates the possibility of using the heart rate indicator not only as a method of biological control of incubation, but also as a method allowing the correction of the incubation regime, based on the heart rate of the embryo.

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