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Monitoring of live chick embryo based on acoustic and vibration signals with a new semi-invasive technology

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Abstract

A new semi-invasive technology is developed in this paper to measure the heartbeat of live chick embryos. A small hole is made by a pin at the top of egg air cell in order to let the heartbeat signal after amplification coming out. Two methods are presented, one is to measure the acoustic signal by installing a microphone over the small hole at the top of air cell of chick embryo (called acoustic signal method), and the other is to measure the vibration signal of a thin tinfoil which is glued on the hole at the top of egg air cell (called vibration signal method). According to the signals measured, we can know the semi-invasive technology not only identifies the live embryo but also distinguishes the strong and weak embryo. By comparison, the signals measured by acoustic signal method and vibration signal method are in good agreement with each other, which demonstrates the validity of semi-invasive technology. The conclusion will lay an important foundation for the further exploring automatic monitoring of chick embryo development.

Keywords: Acoustic and vibration signal methods, Chick embryo, Live identification, Semi-invasive technology.

Introduction

The monitoring of embryo in the period of egg incubation is very important to the development of poultry industries. To identify the life or death of chick embryos is an indispensable process in the incubation. In order to avoid contaminating the environment in which other live embryos are being incubated, the dead embryos (sometimes weak embryos) must be picked out in time. The mainly different characteristics between the live and dead embryos are: 1) the live chick embryo has regular heartbeat, 2) the muscle of live chick embryo has random movement, and 3) there exists gas exchange between live chick embryo and external environment (Khandoker et al., 2003). Owing to the very weakness of characteristic signals of the live embryo, recently five test methods were used to obtain the live information, which were ECG (electrocardiogram), ICG (impedance-cardiogram), BCG (ballistocardiogram), ACG (acoustocardiogram) and lighting method (Hiroshi Tazawa et al., 1999; Hiroshi Tazawa et al., 2002; Ryuichi Akiyama et al., 1999; Kenji Moriya et al., 1999). The ECG and ICG methods were used to measure the characteristic signals of live chick embryo by making a hole on the eggshell and inserting a pair of electrodes. These two methods belong to invasive technology which can damage the biological structure of the chick embryos (Pearson and Hiroshi Tazawa, 1999; CHE Yil et al., 2005; Chiba et al., 2004). The development of chick embryos should be stopped after being measured, so that the two methods could not meet the needs of biological industry. BCG and ACG methods belong to non-invasive technology (Ryuichi Akiyama et al., 1999). The BCG method was used to measure the displacement of the surface vibration of chick embryos (Ono, et al., 1997). The ACG method was employed to measure the acoustic signal from chick embryos in incubation, and the acoustic transducer was fixed over eggshell. In order to decrease the impact of the environment noise on measurement precision, the egg to be measured was putted in a closed container (Haquea et al., 1994; Akiyama et al., 1997). Because of the high stiffness of eggshell, the acquired signals using non-invasive technology were extremely weak and had a very small signal-to-noise ratio. Although lighting method employed light source was widely used, it could not identify the dead chick embryo timely and accurately. Moreover, this method had many disadvantages, such as heavy labor intensity, high false identification rate, etc. According to the equation of fluid dynamics, a novel semi-invasive technology is developed in this paper to measure the heartbeat signal of chick embryo. Using this technology, a small hole at the top of egg air cell is made by a pin in order to let the heartbeat signal after amplification coming out. In the sound signal method, a chick embryo is putted in a tapered container open at one end，and a microphone is installed over the small hole on eggshell to measure the acoustic pressure signal. In the vibration signal method, a piece of thin tinfoil is pasted on the small hole at the top of egg air cell, and the vibration displacement of the thin tinfoil can be measured by displacement transducer. Then the measured signals are analyzed by the LMS noise and vibration analysis system. Finally, the experimental curves of chick embryo can be obtained by computer data analysis system. Compared with the traditional test technologies, this technology is simple and reliable, and can be used to identify the live chick embryo without affecting the development of chick embryo. The research will provide an important foundation for further exploring automatic monitoring of embryonic development.

Results and discussion

When experiments are done, the measuring results will be analyzed and comparison will be made with previously published papers in this section.

Measuring results

After the measurement data is processed by LMS noise & vibration analysis system, Fig.9, Fig.10 and Fig.11 show the measuring results with acoustic signal method, and Fig.12, Fig.13 and Fig.14 show the measuring results with vibration signal method. According to experimental curves, the measuring results can be classified into two groups, one is the signals of live chick embryos and the other is the signals of dead chick embryos. In the experiment based on acoustic signal method, the experimental results of live chick embryo are showed in Fig.9 and Fig.10, and the experimental curve of dead chick embryo is showed in Fig.11. In the experiment on the basis of vibration signal method, the experimental curves of live chick embryo are showed in Fig.12 and Fig.13, and the experimental curve of dead chick embryo is showed in Fig.14. Generally speaking, for the chick embryos incubated in same condition, the amplitudes of the experimental curves should be consistent. The bigger the amplitude is, the stronger the chick embryos are. In other words, the chick embryos corresponding to Fig.10 and Fig.13 are well developed and able-bodied, which shows that the semi-invasive technology not only identifies the live embryos but also distinguishes the strong embryos and weak embryos. Because the two experimental methods are non-contact measurement, they can be widely used to monitor the chick embryonic development. Comparing the results with Liang Sen, et al. (2010), we can find that the signals measured by the displacement transducer and microphone are in good agreement with each other, which demonstrates the validity of semi-invasive technology.

Comparison and analysis

By comparing experimental curves in this paper with invasive technology (Pearson and Hiroshi Tazawa, 1999; CHE Yil et al., 2005; Chiba et al., 2004) and non-invasive technology (Ryuichi Akiyama et al., 1999; Haquea et al., 1994; Akiyama et al., 1997), we can find the semi-invasive technology presented in this paper has many advantages, such as high signal-to-noise ratio, not harming the development of chick embryos, low false identification rate, etc. So this technology can be widely used in reproduction of poultry and long-term automatic monitoring of chick embryo development. By comparing the sound signal method with vibration signal method, we can find the different method has different advantage. However, finding out an executable, effective and economical measuring method is significant for reproduction of poultry as well as scientific investigation. In the acoustic signal method, microphone is used to detect the heartbeat signal of chick embryo and it can also detect the tinny environmental noise. So the testing is carried out in anechoic chamber. In vibration signal method, the measurement can be done in ordinary lab. But many factors can also influence the measuring results, which include egg curvature radius at the detected position, the leak of air cell sealed by the thin tinfoil, the dimension error of the hole on eggshell, etc. Testers should pay more attention to about these. Because the acoustic signal method needs an anechoic chamber, it is suitable for large scale monitoring of the chick embryo in the period of incubation. While vibration signal method is more economical and easy to operate, it is suitable for the scientific research and reproduction of poultry.

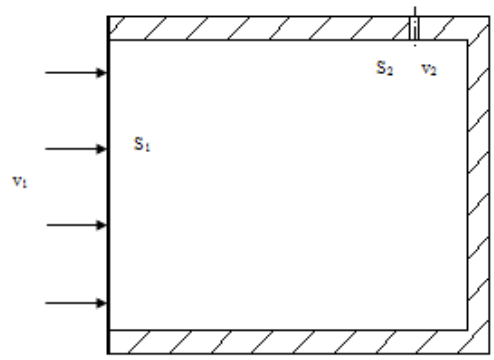

Fig 1. Experiential principle of semi-invasive technology

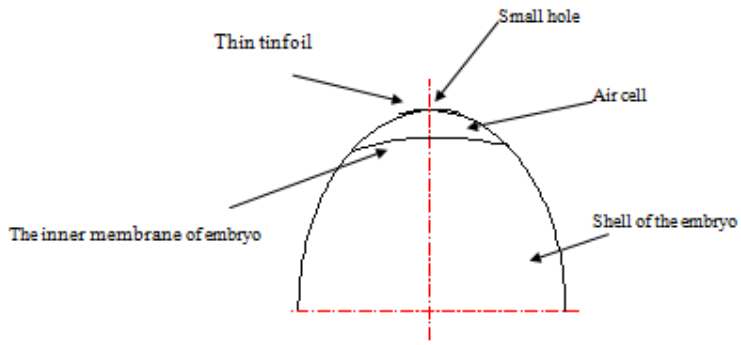

Fig 2. Upper half of the measured chick embryo.

Fig 3. Egg with a hole and egg with a piece of thin tinfoil glued on the hole

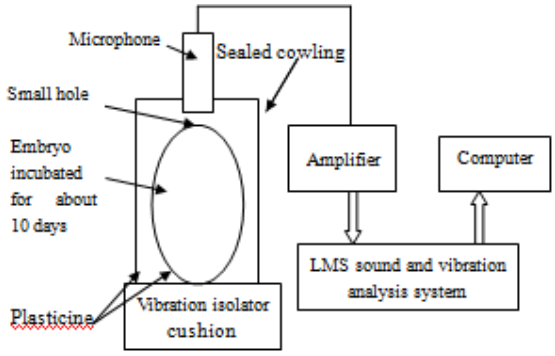

Fig 4. Experimental principle of sound signal method

Materials and methods

In this section, principle of semi-invasive technology, experiment of acoustic signal method and experiment of vibration signal method are developed in detail.

Principle of semi-invasive technology

Experiential principle is shown in Fig.1, which consists of three parts: a rigid box open at one end, a rubber film fixed on the open end of box and a small hole on the box. According to the basic equation of fluid dynamics, a tiny velocity v_1 of the rubber film could cause a huge gas flow velocity v_2 at the small hole. Without consideration of the compressibility of gas, the magnification of speed is an area ratio of rubber film S_1 to the hole S_2. If a piece of thin tinfoil is pasted on the small hole, the small displacement of the rubber film can make a huge motion of the thin tinfoil. By using this principle, the tiny movement velocity of the egg inner membrane caused by the heartbeat of chick embryo can produce a big air flow speed at the hole on the top of air cell. Theoretically, the magnification is an area ratio of the egg inner membrane to the small hole. Practically, the gas in air cell is compressible, so that the magnification is smaller than the ideal value. According to experiential principle above-mentioned, the semi-invasive technology is presented in this paper, two measurement methods to identify the live chick embryo are developed, one is acoustic signal method and the other is vibration signal method. Using this technology, A small hole with 3～5mm in diameter is drilled by a pin at the top of egg air cell. In order to prevent unwanted impact on the further development of chick embryo, the eggshell but not the inner membrane should be punched as shown in Fig. 2 and Fig. 3. Due to the high stiffness of the eggshell, generally the heartbeat signal passing through the eggshell is very tiny. Therefore, the environmental influence makes it very difficult to detect this signal. The intention of drilling hole is to have the air flow signal of egg air cell coming out directly from the hole instead of the air flow signal passing through the eggshell, so that the signal is strong enough for the transducer to measure accurately. By comparison with the traditional non-invasive and invasive technologies, this technology is referred to as semi-invasive technology. The eggs employed in experiments are chick embryos incubated at normal condition for about 10 days and 30 eggs are required in these experiments.

Experiment of acoustic signal method

The test is carried on in the anechoic chamber. The main laboratory equipments used in this experiment include: a microphone (produced by GRAS Company, Denmark), LMS sound and vibration analysis system, an experimental bench, a self-made tapered container open at one end and a self-made vibration isolation cushion. The experimental principle presented is showed in Fig.4. In order to reduce the impact of the environmental factors on the experimental results and ensure the precision of the experimental data, testing should be carried out in anechoic chamber. A self-made tapered container open at one end and a self-made vibration isolation cushion which is a sandwich structure made up of a piece of viscoelastic damping film and two pieces of sponges are used in order to minimize the impact of environmental factors on experimental results. The stainless steel plate putted on the vibration isolation cushion is to reduce the influence of the vibration isolation cushion itself on sound signal. In testing process, the chick embryo to be measured is putted in the tapered container and fixed on stainless steel plate by plasticine. The joint between the open end of the tapered container and the

Fig 5. Signal acquisition and analysis system

Fig 6. Experimental device of sound signal method

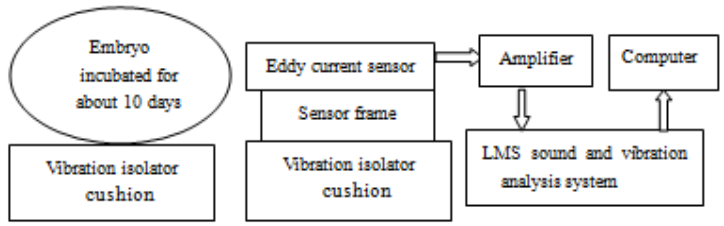

Fig 7. Experimental principle of vibration signal method

Fig 8. Experimental device of vibration signal method

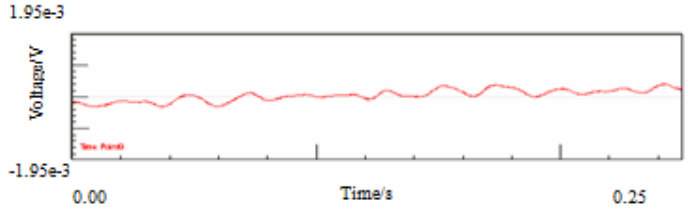

Fig 9. Measuring result of weak chick embryo using acoustic signal method.

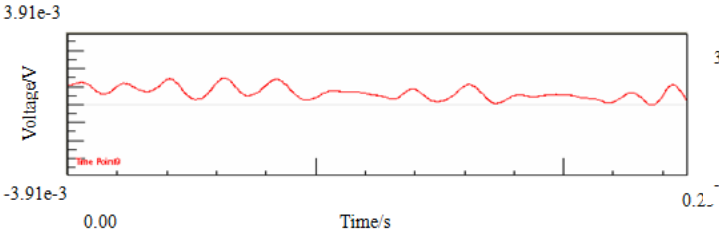

Fig 10. Measuring result of strong chick embryo using acoustic signal method

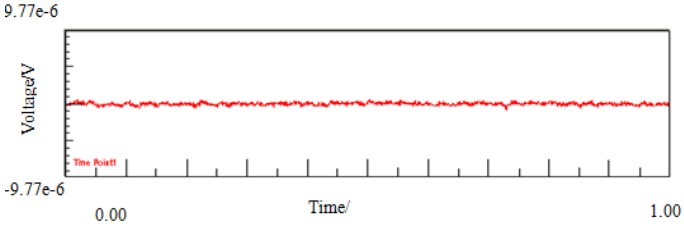

Fig11. Measuring result of dead chick embryo using acoustic signal method

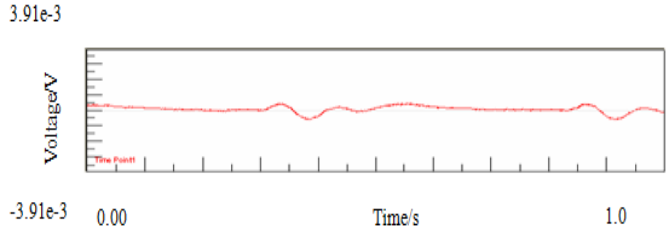

Fig12. Measuring result of weak egg embryo using vibration signal method

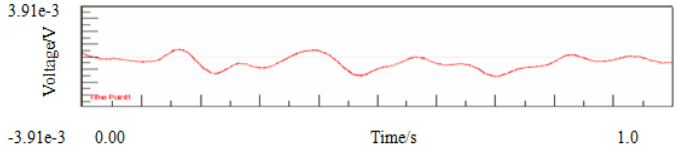

Fig 13. Measuring result of strong egg embryo using vibration signal method

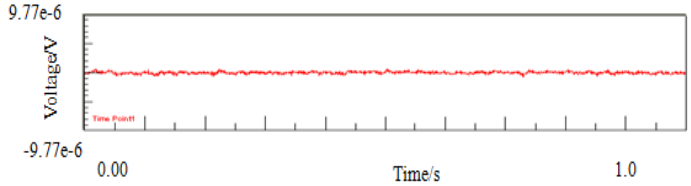

Fig 14. Measuring result of dead egg embryo using vibration signal method

stainless steel plate is required to be sealed by plasticine. The microphone is inserted into a hole on the tapered container, faces to the hole at the top of egg air cell and is in alignment with the hole on the eggshell. The distance between microphone and eggshell should be adjusted in 3 ~ 5mm. The vibration of egg inner membrane is driven by the heartbeat of chick embryo, which can change the volume of air cell. Because the diameter of the hole at the top of egg air cell is tiny, a small movement of egg inner membrane could cause an intense air flow at the hole on the eggshell. Consequently the sound pressure around the small hole will have a great change, and sound transducer can measure this change accurately. Fig.5 shows the signal acquisition and analysis system, and Fig.6 shows the testing device.

Experiment of vibration signal method

The measurement of vibration signal of the chick embryo is carried out in ordinary laboratory. The main laboratory equipments used in this experiment include: an eddy current sensor (produced by OUDAN instrument, Shanghai), LMS noise and vibration analysis system, an experimental bench, self-made sensor bracket and self-made vibration isolation cushion (the same as above-mentioned). Principle of experiment presented is showed in Fig.7. Because the eddy current sensor can only detect the displacement of conductor, a piece of thin tinfoil should be glued on the hole of eggshell before measurement. The dimension of the thin tinfoil is two times as large as that of the probe of eddy current sensor. The small hole at the top of egg air cell should be completely covered and sealed by the thin tinfoil to ensure the precision of experimental results. The chick embryo to be measured is laid on the vibration isolation cushion, the relative position between the chick embryo and the probe of the transducer should be adjusted to make sure the probe is in alignment with the hole on the eggshell, and the distance between the probe and the hole should be kept in 0.5~1.5mm. In order to minimize the impact of environmental vibration on measurement data, the self-made vibration isolation cushion as above-mentioned is used here to isolate the measurement device from the vibration of the experimental bench. The vibration of the inner membrane is driven by the heartbeat of chick embryo as showed in Fig.2.

If the pressure of the egg air cell changes, the vibration of thin tinfoil on the hole will follows this change. When the heartbeat driving the inner membrane movement shrinks, the pressure in air cell will decrease and the thin tinfoil as showed in Fig.3 will move downward. Otherwise, it will move upward. Because the stiffness of the thin tinfoil is much smaller than that of the eggshell, a small movement of egg inner membrane could lead to a bigger vibration of the thin tinfoil. Therefore the displacement of the thin tinfoil is strong enough for the eddy current sensor to measure accurately. The signal acquisition and analysis system in this experiment is showed in Fig.5 and the testing device is showed in Fig.8. The measurement can be started after the system adjusted becomes stable.

Conclusions

The extraction of characteristic information in the period of egg incubation plays a very important role in the development of poultry industries. On the basis of traditional testing technologies, a new semi-invasive technology is proposed in this paper. A small hole is made by a pin at the top of egg air cell in order to let the heartbeat signal after amplification coming out. Two measuring methods using this new technology are presented, one is the acoustic signal method, and the other is the vibration signal method. By using these signals measured by these two methods, we can know whether the embryo is alive or not. So the methods using this new technology are of great significance for reproduction of poultry and long-term automatic monitoring of chick embryo development.

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