

## Original papers

## Cardiac signal behavior of early and late hatch chick embryos during incubation

Khaliduzzaman<sup>a,c,\*</sup>, Shinichi Fujitani<sup>b</sup>, Naoshi Kondo<sup>a</sup>, Md Syduzzaman<sup>a</sup>, Afzal Rahman<sup>a</sup>, Tetsuhito Suzuki<sup>a</sup>, Yuichi Ogawa<sup>a</sup>, Ayuko Kashimori<sup>b</sup>, Tateshi Fujiura<sup>a</sup>

<sup>a</sup> Laboratory of Bio-Sensing Engineering, Kyoto University, Kyoto 606-8502, Japan

<sup>b</sup> NABEL Co., Ltd., Kyoto 601-8444, Japan

<sup>c</sup> Faculty of Agricultural Engineering and Technology, Sylhet Agril. University, Sylhet 3100, Bangladesh

## 1. Introduction

Chicken became the largest source of global meat and a vital source of reasonably priced protein by overtaking pig meat production in 2017. To meet this growing demand, the poultry production must be efficiently scaled up. Thus, precision poultry farming, in particular high-quality chicks, will play an essential role.

Nowadays, the supply of high quality and uniform batches of chicks is considered as one of the most important challenge to breeders and poultry farmers (Decuyper et al., 2001). However, the homogeneity of the day-old chick cohort is frequently compromised by a wide-spread hatch window; it contains chicks which have had short to long incubation periods (Bergoug et al., 2013; Tona et al., 2003). This negatively affects that cohort's post-hatch performance. It is well known that late hatch chicks show extensively inferior quality (growth rate, mortality and disease susceptibility) in post-hatch performance (Løtvedt et al., 2014). Moreover, late hatch chicks cause many downstream complications in post-hatch chick sorting, feed and water supply, vaccination and maintenance of the rearing environment relate to their hatching time differences (24–48 h) from those of the early hatch chicks.

Although the linkage between embryo growth, hatching time and day-old chick uniformity are known through studies of the effect of incubation temperature, it is less clear how physiological variability in embryo growth effects the timing of hatching (Lourens et al., 2005; Noiva et al., 2014). For instance, how the cardiac activity of chick embryos during incubation affects the hatch window has not yet been studied. The heart is a vital organ in chick embryos, which plays an important role in embryonic development during incubation. An embryo's heart undergoes complex changes throughout the developmental stages of incubation. These cardiac changes are suspected to influence the growth of the embryo and subsequent hatch window. According to Ar and Tazawa, avian embryos may stay in the egg for a fixed period and all embryos have a more or less constant total heart beat during their incubation span (Ar and Tazawa, 1999). Thus, the growth of the

embryonic heart could be a good indicator of chick embryonic growth and maturity. Hence, higher cardiac activity during incubation may shorten the hatching time of chick embryos.

In oviparous organisms, the duration of incubation can be a critical life history variable (Du et al., 2009). Hence the embryonic development history of chick embryo during the incubation period might have significant importance for precision hatching and post hatch chick performance. In these circumstances, a cardiac physiological study of various hatch groups especially late hatch chick embryos is highly important on the field of developmental physiology and precision poultry production system. Early detection during incubation of potentially late hatch embryos could significantly contribute to the humane treatment of chicks (late hatch chicks are discarded) and production efficiencies (minimize labor, energy and space utilization).

Visible transmission spectroscopic method cannot be used to monitor hatching eggs at the second half of incubation due to very low transmittance (Kemps et al., 2010). Besides, egg shell has high absorbance in the UV–Vis region. Therefore, near-infrared (NIR) could be a good option to study hatching egg and quantify dynamic activity of chick embryo as NIR has higher transmission in hatching egg even second half of incubation.

Therefore, the present research was designed firstly to investigate the behavioral pattern of cardiac activity of various hatch groups non-invasively during incubation period using near infrared (NIR) sensor and secondly, to separate late chicks from late hatch chicks based on their cardiac activity signal.

## 2. Materials and methods

## 2.1. Materials

This research was carried out in strict accordance with the animal experiment regulations of Kyoto University (Animal experiment approval number: 28–59). A total of 51 light brown eggs laid by a 54-week old parent flock (ROSS 308 strain, Japanese name “Chunky”) from a

\* Corresponding author at: Laboratory of Bio-Sensing Engineering, Kyoto University, Kyoto 606-8502, Japan.  
E-mail address: [khaliduzzaman.88s@st.kyoto-u.ac.jp](mailto:khaliduzzaman.88s@st.kyoto-u.ac.jp) (Khaliduzzaman).

commercial poultry hatchery (Yamamoto Co. Ltd., Kameoka, Kyoto, Japan), were selected based on major diameter ( $59.5 \pm 3.0$  mm), minor diameter ( $46 \pm 1.0$  mm), mass ( $68 \pm 5.0$  g) and shell color (red ration,  $r = 0.375 \pm 0.015$ ), for incubation studies. Uniformly graded fertile eggs were used to minimize heterogeneity among the embryos, hence to reduce the effects of zootechnical parameters on hatch window. Prior to incubation, all eggs were stored for 6 days at  $18.0 (\pm 0.5)^\circ\text{C}$  and  $80 (\pm 5)\%$  of relative humidity (RH). A color image analysis method was performed to sort egg shell color using RGB color space.

$$r = \frac{R}{R + G + B} \quad (1)$$

where  $R$ ,  $G$  and  $B$  are the red, green and blue components of an egg image respectively.

One incubator (SSH-02, Showa Furanki, Saitama, Japan) consist of three egg trays was used for incubation of fertile eggs ( $20 + 20 + 11 = 51$ ) at  $37.8^\circ\text{C}$  and  $55\%$  RH (Lourens et al., 2005). Prior to setting the eggs into the incubator, eggs were preheated for 16 h (first 6 h at  $28^\circ\text{C}$  and for the remaining 10 h at  $30^\circ\text{C}$ ) to reduce thermal shock on blastoderm and to reduce the early embryo mortality. After day 18, the eggs were transferred to hatcher three trays maintaining temperature at  $37.8^\circ\text{C}$  and  $60\%$  RH. The embryo cardiac signal of all incubated eggs was measured using the near-infrared sensor from day 8 to 19 of incubation.

## 2.2. Near infrared sensor

An Embryonic Vital Scope (EVS), hereafter referred as an NIR sensor consists of six light emitting diodes (LEDs) that emit light of  $870$  nm and a photodiode that receives the light passing through the egg. The light received is converted into current by the photodiode that is further converted into a voltage signal by amplification using a trans-impedance amplifier (Figs. 1 and 3). The average output voltage was maintained between  $3.00$  and  $9.76$  V (V). If the LED light was not enough to reach  $3$  V, the input current was increased by adjusting resistance automatically. This happens when the embryo inside the egg starts becoming larger at the latter half of the incubation. The sampling rate per second of the signal was  $33.3$  (sampling points per second), which was determined by the LED emission cycle ( $30$  ms). As the Nyquist frequency of this sensor was  $16.5$  Hz (Hz), the sensor can be used for any movements less than or equal to  $5$  Hz. Fluctuations due to embryonic movements are normally  $1.0\%$  of the average output voltage and center around the mean value (Fig. 2). Therefore, the part of the

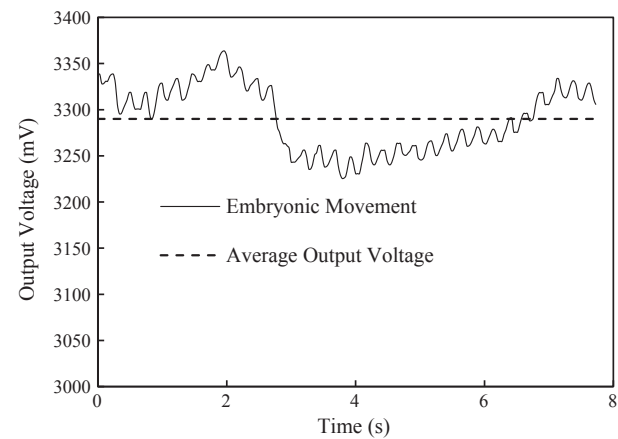


Fig. 2. Diagram of time domain output signal of NIR sensor explaining embryonic movement as signal fluctuation around the average output voltage.

signal assigned to embryonic movements was normalized by dividing by the average output voltage, as output voltage varies with the egg sample due to the variation in transmittance.

## 2.3. Signal acquisition

From incubation day 8 to 19, eggs were taken out from the incubator every 24 h for 9 s during which the signal (10 eggs in one tray) was acquired in vertical optical configuration (Fig. 3). To minimize the exposure time of the egg outside the incubator, eggs were immediately placed back into the incubator after the measurements. However, the temperature of the sample eggs outside the incubation were carefully maintained properly by using warm electric blanket to avoid the influence of ambient temperature on activity signal of embryo.

## 2.4. Signal processing and data analysis

The voltage signals obtained by the NIR sensor were transformed into the frequency domain using a Fast Fourier Transform (FFT) of 256 sampling points equivalents to  $7.72$  s for peak frequencies and power spectrum (Fig. 7). The high-frequency peak represents the cardiac movement of the chick embryo whereas the low-frequency peak represents embryo body movement (Fig. 7). A similar approach has also been applied to extract cardiac pulse by other researchers (Khaliduzzaman et al., 2016; Youssef et al., 2014). Body movement is

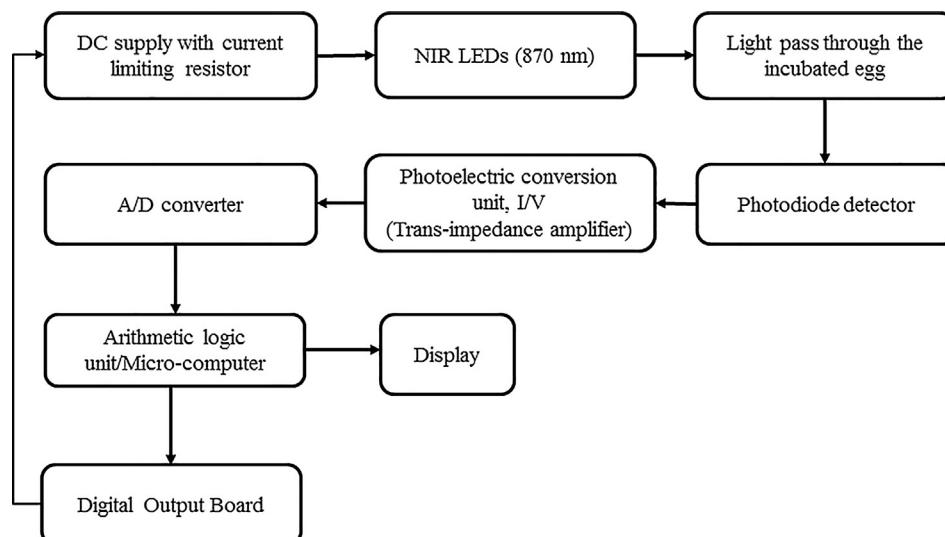


Fig. 1. Flow diagram of working principle of EVS called NIR sensor. DC (direct current), A/D (analogue to digital), I/V (current to voltage).

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