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ABSTRACT Some unresolved questions in poultry science were addressed: what determines the yield of chick embryos or hatchlings; what kind of influence does egg yolk content have on embryonic development; and how to detect eggs producing super grade chicks? Since the yolk acts as a vital energy and nutrient reservoir for embryos, we hypothesized that a higher yolk content of similar sizes eggs would play an important role in embryo or chick viability during incubation, as well as at hatch. As experimental sample, we used ROSS 308 (broiler line) and a nondestructive spectroscopic absorbance method. The influence of high yolk content to embryonic heartbeat and chick yield (i.e., chick weight/egg weight) were then investigated. Embryonic heartbeat signal was measured indirectly using a prototype near-infrared sensor during incubation period. A positive influence was found in both cases. Similar size eggs with higher yolk content were found to significantly (P-value < 0.05) promote higher chick yield at hatch. This methodology may have the potential to be used to precision poultry production system, ornithology, developmental, or evolutionary biology in the near future.

Key words: yolk absorbance, optical sensor, chick yield, super grade chick, animal welfare

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INTRODUCTION

There are 7.8 billion people in the world in 2020, by 2030, this figure will be more like 8.5 billion and by the year 2050, there will be an estimated 9.8 billion people on the planet (United Nations, 2017). To feed this increasing number of people will be quite a challenge in the next several decades. The poultry sector will face perhaps some of the greatest challenges and opportunities of all. This is because poultry meat consumption is rapidly increasing compared to other sources of animal and fish protein, such as beef, pork, wild fish, cultured fish, and lamb, due to its easy access, lower price and acceptable to all religions. To meet this growing demand and make it sustainable, the poultry production must be efficiently scaled up. In industrial perspective, early detection of hatching eggs producing high/super grade chicks might solve many challenges of current poultry industry such as higher productivity, improved hatchery management, profitability, and ethical issues. This could also contribute in minimization the culling of undesired (ie, low grade) chicks.

Separately, some historically unsolved questions in egg and poultry research have great importance to solve: what (e.g., egg albumin or yolk) determines the viability (e.g., chick yield or qualitative score) of chick embryos or chicks; what impact does egg yolk content have on embryonic development; and how to detect super grade chick embryos for next generation egg and poultry industry which have higher quality and growth potentiality? We hypothesized that a higher yolk content would play an important role in embryonic development potentiality during incubation, as well as development at hatch (e.g., higher conversion ratio [**CR**]), since the yolk acts as a vital energy and nutrient reservoir for the development of embryos.

Researchers found that post-hatch growth performance is influenced by the chick yield at hatch (i.e., chick weight/egg weight), breed and strain but not by the egg size (Iqbal et al., 2017). But currently there are no research have been conducted till now nondestructively on how to solve the above raised questions and how to get the super grade eggs producing super grade embryos/chicks. This is probably due to the insufficient/lack of non-destructive methodologies in poultry (hatching eggs) research.

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To answer the above questions, we need to identify eggs with higher yolk content nondestructively and then chick evaluation parameters after hatching (e.g., CR, chick size etc). Because we hypothesised that egg containing higher amount of yolk may produce super grade chicks, since yolk is considered as a main reservoir of energy for embryonic development. The spectroscopic method can predict yolk information without breaking the egg based on relative absorbance of eggs in near-infrared (**NIR**) regions, 832 to 840 nm and 872 to 875 nm (Syduzzaman et al., 2019). Therefore, the objective of this research to identify super grade eggs/chicks even before incubation based on relative absorbance of egg.

MATERIALS AND METHODS

This research was conducted according to the animal experiment guidelines animal of Kyoto University, Japan.

Materials

Eggs from broiler breed (ROSS 308) were used in this experimental protocol. The eggs were received from a hatching eggs producer (Yamamoto Ltd., Kyoto, Japan). Eggs were graded based on major axis (59.5 ± 3.0 mm), minor axis (46 ± 1.0 mm), mass (68 ± 5.0 g) and eggshell color (red ration, r = R/RGB = 0.375 ± 0.015). Prior to incubation, all eggs were temporary stored at 18°C and 75% relative humidity. The eggs were color sorted based on computer vision system using the RGB color space (Islam et al., 2017; Khaliduzzaman et al., 2019).

Eggs Spectral Measurement

For spectral acquisition, a halogen lamp (FHL-10, Asahi Spectra Co., Ltd., Japan) was used as a lighting source and Photonic Multichannel Analyzer (PMA 12 Spectrophotometer, Hamamatsu Photonics, Japan), was used as a spectrometer (Figure 1A and 1B). The relative transmittance of egg sample was measured over the spectral range of 200 to 900 nm. An average of 10 measuring scans was considered for each egg. The exposure time was determined based on the intensity of the detector's saturation point. When exposure was changed, a reference spectrum was rescanned. Moreover, dark current was measured to avoid any electrical noise in the signal and automatically eliminated from the spectra. Spectral acquisition was performed inside a box coated with black color to avoid any outside light.

A Teflon block (30-mm thick) made by polytetrafluoroethylene (PTFE) push rod Ø45 mm, Chukoh chemical Industries Ltd., Japan, was used for acquisition of reference spectra (I_0), which was further used to calculate relative transmittance (T) of spectra from the raw spectra (I). The relative transmittance and absorbance were calculated using the following equations Eqs. 1-2.

$$\text{Relative transmission } (T) = I/I_0 \tag{1}$$

$$\text{Relative absorbance } (A) = \log(1/T) \tag{2}$$

Embryonic Cardiac Performance Measurement

A single wavelength (870 nm) based NIR optical sensor was used for embryonic HB signal acquisition during incubation (Figure 1C). The device consisted of 6 LEDs (Model: L870-04-35, Epitex, Japan) and a photodiode detector (Model: S9269, Hamamatsu Photonics, Japan) together with an amplifier that detected the transmissive light passing through the egg. The input LEDs current (0.73−96.15 mA) was controlled by variable series resistances to keep output signal (volt) within the desirable range (3−9.7 V). The vital signals of all incubated eggs were measured using NIR sensor from incubation day 8 to 18 after taken out from the incubator once daily. To minimize the exposure time of eggs from outer environment, eggs were immediately returned to the incubator after completion of the measurement. The voltage signal obtained by the above optical sensor was transformed into the frequency domain using fast Fourier transformation of 256 data points equal to 7.68 s signal (Figure 3A and 3B). The area under the high-frequency peak was calculated to measure signal energy, which represents heartbeat strength using trapezoidal numerical integration (Khaliduzzaman et al., 2018).

Hatchling Groups

Since CR of all chick hatchlings were varied from 0.68 to 0.88, we categorized the hatchlings into high (>0.77, $n = 26$), medium (0.73−0.77, $n = 13$) and low (<0.73, $n = 7$) CR or yield groups based on median value. The CR means the hatch mass as a percentage of egg mass (Eq. 3). It is also called chick yield at hatch. The CR indicates how efficiently stored resources of an egg is utilized to produce chick hatchling where higher yield is preferred in poultry hatchery practices.

$$\text{Conversion ratio } (R_{CR}) = M_c/M_e \tag{3}$$

where Mc is the mass of chick or hatchling and Me is the mass of egg before incubation

To see the statistically significant differences at P-value <0.05, 2-sample t test was applied between higher and lower CR groups.

RESULTS AND DISCUSSION

Relative Absorbance and Chick Yield

Chicks group with higher CR (i.e., higher yield group) showed significantly higher average relative absorbance of

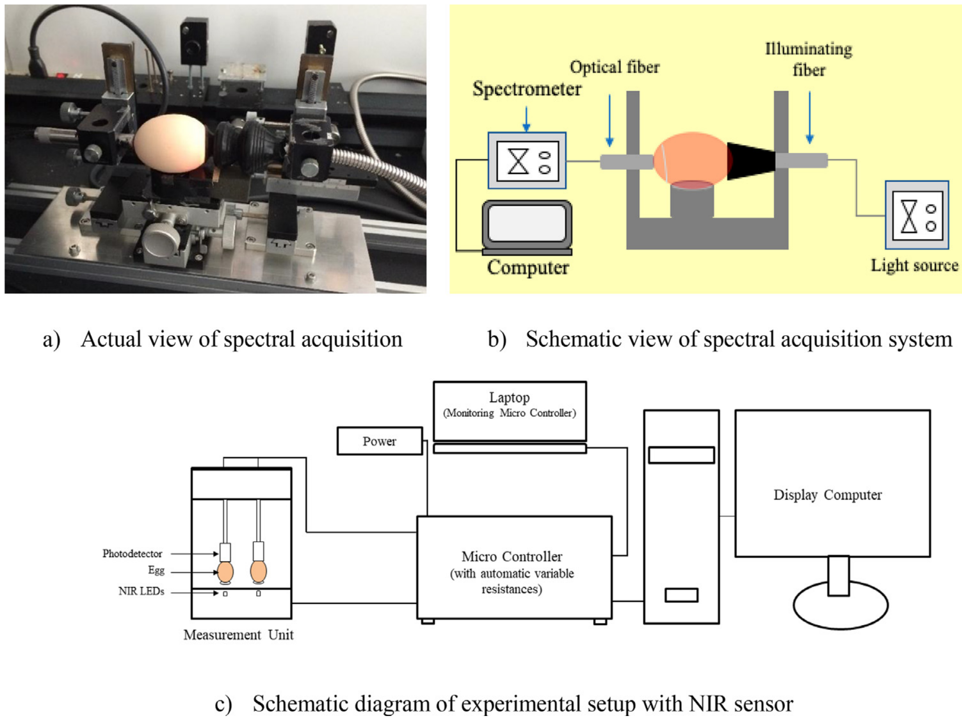


Figure 1. Actual and schematic views of instrumental setup of the spectral acquisition system and heartbeat signal acquisition system.

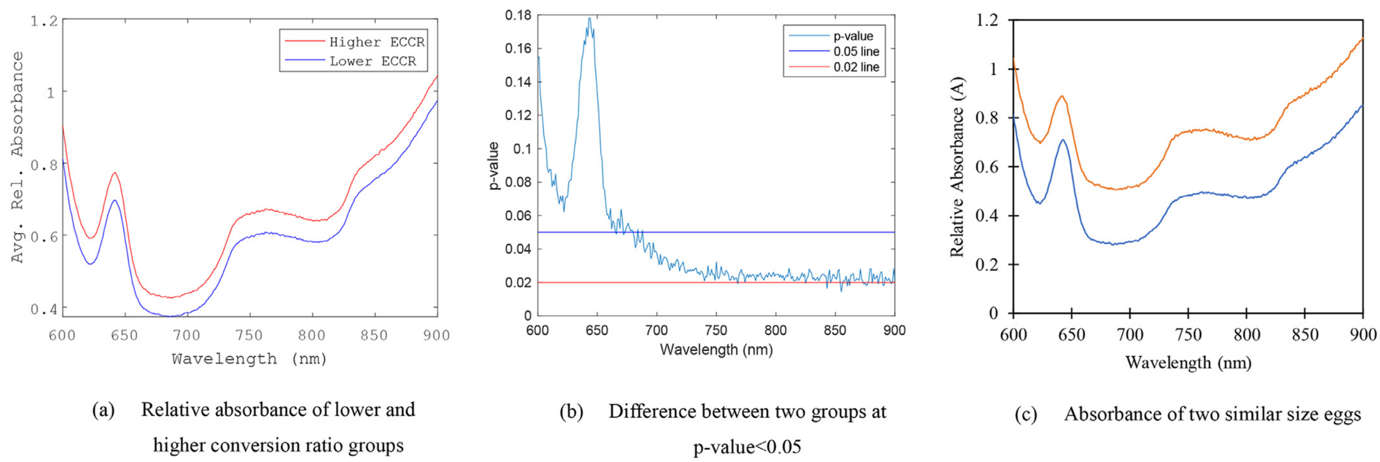


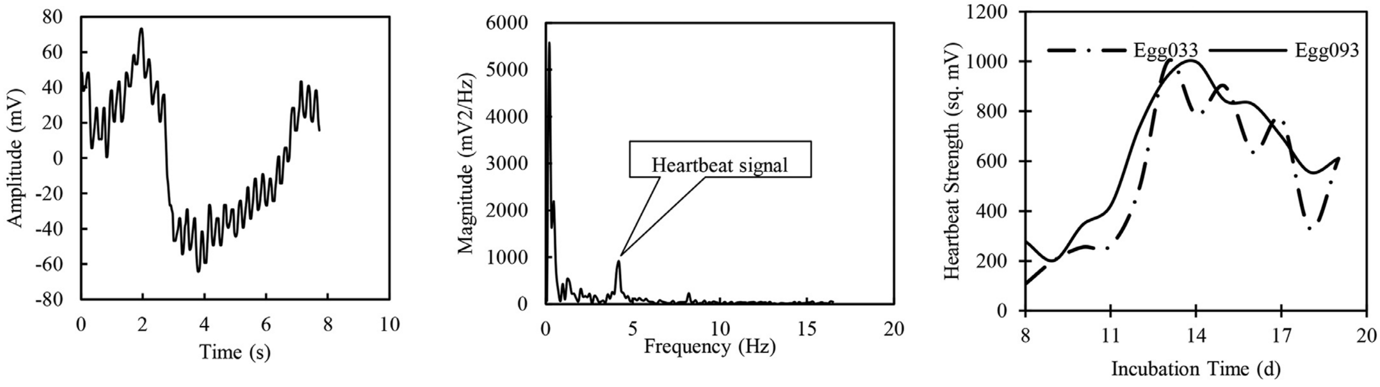
Figure 2. Differences in average relative absorbance of higher and lower chicks yield groups. (A) Relative absorbance of lower and higher conversion ratio groups, (B) higher chick yield showed significantly (P-value < 0.05) higher relative absorbance of eggs, and (C) relative absorbance of two very similar size eggs from two groups. Higher absorbance value indicated higher yolk content. ECCR referred to egg to chick conversion ratio or simply conversion ratio.

non-incubated eggs as shown in Figure 2. Higher absorbance of intact egg in the wavelength of 600 to 900 nm indicates higher yolk content of egg (Syduzzaman et al., 2019).

Although generally bigger size egg contains larger amount of yolk, this infleunce is not always depending on size of the egg. The variation in yolk amount can exist independent of egg size. Because two similar size eggs can have difference to their amount of yolk content based on their individual variation. This difference between two similar size eggs was clearly visible in Figure 2C. Therefore, the differences we found in relative absorbance of two similar size eggs indicated their differences in their yolk contents. Some eggs may possess higher amount of yolk and lower amount of albumen and vice-versa. These variations may arise from individual traits of bird, feeding and nutrition, gender specific maternal investment, and genetic factors.

The CR (i.e., chick weight at hatch/ egg weight) was influenced by relative absorbance of egg in NIR region (760−900 nm). Eggs with higher relative absorbances before incubation, indicating higher yolk amount or ratio, produced chicks with higher CR. For this reason, higher conversion (ie, higher yield) group had significantly (P-value <0.005) higher relative absorbance indicating larger yolk content than lower conversion group of chick hatchlings.

We found an interesting relation between yolk content (i.e., higher relative absorbance) and chick size. Egg



(a) Time-domain HB signal day 15 (b) Frequency-domain signal at day 15 (c) Difference in HB strength

Figure 3. Typical cardiac rhythm in time-domain and frequency domain and cardiac performance in terms of heartbeat strength of two typical embryos from two similar size eggs during incubation where egg id `Egg 033` represented lower relative absorbance group and egg id `Egg 093` for higher relative absorbance groups or CR groups.

with larger yolk produced bigger chick. Perhaps, the reason is that bigger egg may have bigger size yolk resulting bigger chick size. However, similar size egg with bigger yolk also produced bigger chicks. Since we know that the yolk acts as a vital energy and nutrient reservoir for embryos, thus it played an important role in embryonic growth by providing a favourable environment (e.g., resources). This findings can be interpreted with the statement by Iqbal et al. (2017). They reported that egg weight (i.e., size) does not affect significantly the albumen and yolk ratio. The differences in yolk to albumen ratio is influenced by genetic factors and individual traits. Since eggshell provides major minerals to developing chick embryos during second half of incubation, chick CR also may be influenced by eggshell quality parameters (e.g., thickness, density) which were not considered due to lack of available nondestructive technology. Considering shell factors might increase the visibility of differences between super grade and low grade eggs or chicks groups.

Variation in Cardiac Performances

The chick embryos produced from higher relative absorbance group showed higher cardiac performances and more stable signal strength during incubation (Figure 3). The strength of the heartbeat signal was stronger for super grade (i.e., higher yield group) chick embryos than lower grade chick embryos during incubation. The larger signal strength might be come from larger heart size, since yolk plays an important role in organ development in developing chick embryos during incubation process.

This research revealed that egg relative absorbance in NIR region indicating yolk content of an egg was vital factor which is closely associated with embryonic development (e.g., chick yield at hatch, and heartbeats strength). The egg which contained higher amount of yolk ratio (i.e., higher absorbance in certain wavelengths) produced bigger size chick due to their higher CR (i.e., higher chick yield at hatch) as shown in Figure. 2A. This may also produce healthy hatchlings which may act as a game changing tools for next generation hatchery management and poultry industry. Petek et al. (2010) reported a positive relation of chick weight and chick length with subsequent growth performance in broiler. Thus, this research finding may also contribute to animal welfare issue by avoiding incubation of egg with lower yolk absorbance (e.g., low-grade eggs that may produce low-grade chicks which could be discarded in hatchery practices).

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Author Contribution: A. K. (Alin Khaliduzzaman) designed and conducted the experiments and A. K. (Ayuko Kashimori) assisted the experiments. A. K. (Alin Khaliduzzaman) made a major contribution in preparing the manuscript and data analysis. Y. O. and T. S. provided the technical guidance. N. K. provided the research direction and was host researcher of JSPS postdoctoral researcher (Alin Khaliduzzaman).

DISCLOSURES

The authors declare no conflict of interest.

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