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Detection of infertile eggs using visible transmission spectroscopy combined with multivariate analysis

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ABSTRACT

Visible transmission spectroscopy combined with multivariate analysis was used to develop a nondestructive detection system for hatching egg fertility. The transmission spectra (500–750 nm) of 165 light brown-shell broiler hatching eggs were acquired between incubation time 0–144 h at 24 h interval. Several multivariate classification methods, i.e. k-means clustering, linear discriminant analysis (LDA) and support vector machine (SVM) were used to discriminate between fertile and infertile eggs. The LDA and SVM classification models achieved 100% classification accuracy for both fertile and infertile eggs, while the k-means clustering model had a classification accuracy of 96% for fertile eggs and 100% for infertile eggs at 96 h of incubation. These results demonstrate that visible transmission spectroscopy, combined with an appropriate multivariate method, has the potential to use for detection of infertile eggs in commercial hatchery operations.

1. Introduction

Poultry production plays an important role worldwide in the provision of essential dietary protein through both egg and meat production. In this production chain the continuous supply of 1-day old chicks to poultry farmers is a very important component to meet the increasing demand for this meat and eggs. In turn, the efficient supply of these 1-day old chicks depends on the percentage of fertile eggs that are successfully hatched.

Poultry hatchery statistics indicate that about 5–20% of all eggs put into incubation are infertile. Unproductive incubation of such infertile eggs results in loss of energy, excessive use of incubator space, increased time and handling costs. Therefore, to improve the efficiency of the process, it is important to detect and remove infertile eggs at an early stage of incubation, and thereby maximize the percentage of incubated eggs that are fertile.

Currently, to partially achieve this, a candling technique is widely used in the poultry industry to remove infertile eggs or dead embryos from the incubator. In this candling system, candlers have to manually examine individual egg, which is laborious and time consuming. Moreover, in this continuous operation human error resulting from fatigue is a problem. Since this candling is a time and labor consuming process, only 5% of eggs are candled between incubation days 7 to days 10 to determine flock fertility. As a result, most of the infertile eggs remain in the incubator until day 18.

The presence of these infertile eggs or dead embryos in the incubator is likely to harbor and provide a source of pathogenic contamination to other eggs. Therefore, it is important to develop an efficient, accurate, non-destructive and automated system for the early detection and removal of infertile eggs. Detection and removal of most of the infertile eggs at an early stage of incubation would benefit the poultry hatchery by reducing incubator space usage, energy, handling costs and preventing pathogenic contamination to other eggs.

Several machine vision and other approaches have been reported for the determination of fertility or detection of early embryo development in hatching eggs. Danno et al. (1979) used a thermal imaging technique to discriminate between fertile and infertile eggs based on the temperature difference that exists

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between them at day 4 of incubation. However, egg shell temperature difference between fertile and infertile egg at incubation day 4 is negligible and is not useable as discriminating parameter. Later, Das and Evans (1992a, 1992b) developed a machine vision system to detect fertile eggs in white-shell eggs. They combined image histogram characteristics with neural network classifiers, and reported that fertile and infertile eggs at day 3 and day 4 of incubation could be discriminated with an accuracy of over 93%.

Recently, a hyperspectral imaging technique has been used to detect hatching egg fertility and embryo development (Lawrence et al., 2006; Smith et al., 2008). Lawrence et al. (2006) applied the hyperspectral imaging technique for detecting embryo development of hatching eggs during the first three days of incubation. They used Mahalanobis distance (MD) classification and partial least square regression (PLSR) methods to develop a prediction model from hyperspectral images of the hatching eggs. The Mahalanobis distance (MD) based classification model achieved a 100% accuracy at day 2 and day 3 of incubation. However, they used only fertile eggs and number of samples was very small (N = 24). Later, Smith et al. (2008) applied the hyperspectral imaging technique to detect broiler hatching egg fertility and embryo development prior to hatching. They developed a predictive modeling technique for determining fertility and embryo development during the first three days of incubation. However, the prediction model produced much lower classification results, both on validation data (63–83% accurate) and verification data (50.8% accurate). The main drawbacks of these machine vision systems are it is costly to acquire images and processing them is complex.

An acoustic resonance analysis method has been applied to the detection of embryonic development in hatching eggs (Bamelis et al., 2002). The authors claim that differentiation of fertile and infertile eggs is possible due to the vibration behavior of fertile incubating eggs, which suddenly changes after 100 h of incubation, while infertile egg do not show this behavior. From a practical point of view, this method is not applicable because of the large variability in resonance frequency between eggs.

Furthermore, Bamelis et al. (2002) used visible light transmission spectroscopy to detect embryo development in hatching eggs. The ratio of transmitted light at 577 nm (a hemoglobin-sensitive waveband) and 610 nm (a reference waveband) was used as a measurement of blood in hatching egg. Average value of the ratio (T577/T610) for fertile egg group and infertile egg group were plotted on a 2-D graph and from the time series curve it was reported that embryonic development could be detected after 108 h of incubation. However, no attempt has been made to develop classification model for detection of hatching egg fertility. Moreover, the use of a single wavelength to detect fertility of hatching egg might limit the accuracy of a classification model.

Therefore, the goal of this study is to develop a hatching egg fertility detection model based on spectral information combined with appropriate multivariate analysis technique. First, principal component analysis (PCA) technique was applied to extract information from the visible transmission spectra for accurate discrimination of fertile and infertile eggs. Later, multivariate classification model was developed for detection of hatching egg fertility.

2. Material and methods

2.1. Material

A total of 165 light brown-shell chicken eggs laid by a commercial broiler breed (Ross 308 strain) were collected from a commercial poultry hatchery (Yamamoto Co. Ltd., Kameoka, Japan). In order to minimize the effect of egg shell pigmentation on transmission spectra, eggs with similar shell color (selected by color image analysis method) were selected. In addition, all samples were selected within a range of 43.5 (±1.0) mm (diameter), 56.5 (±2.5) mm (height) and 60.5 (±5.0) g (weight), to obtain nearly homogenous samples. Prior to incubation, all eggs were stored for 3 days at 15.0 (±0.5) °C and 80 (±5) % relative humidity (RH).

2.2. Spectral acquisition

The experimental setup used for the measurement of the transmission spectra of the intact egg is shown in Fig. 1. An illuminating fiber was used for cool illumination of the eggs and the optical fiber was used to collect and transport the transmitted light to the spectrometer. A Hamamatsu C 7473-36 model spectrometer (Hamamatsu Photonics K. K, Japan) was used to acquire spectral transmittance and a halogen light source (FHL-10, Asahi Spectra Co. Ltd., Japan) was used for illuminating the samples. This light source consisted of a dichroic reflector type halogen lamp (capable of cutting off infrared energy) in order to prevent warming of the egg surface where the light beam is shone onto the eggshell. The software package PMA-11 Spectral Analyzer for windows (PMA Software U6039-01, Hamamatsu Photonics K.K., Japan) was used to control the spectral acquisition.

The transmission spectra of the eggs were measured over the spectral range of 200–950 nm at 1 nm intervals. The integration time for one scan was 100 ms and the spectra of each egg consisted of an average of 10 scans by keeping the eggs in same place to minimize within egg variability. Since the characteristics of halogen lamps change over time, the spectrometer was calibrated before each measurement using a Teflon block of 30 mm thickness (Kemps et al., 2010). In addition, the reference spectra were measured after each 10 samples to evaluate any changes in the reference spectra with time. Furthermore, a correction was made for electrical noise by taking the spectra with no light exposure in the spectrometer. All measurements were done inside a black box to shield any stray light from entering. The raw transmission spectra of the samples were transformed into the relative transmission (T) using Equation (1).

$$T(\lambda) = \frac{S(\lambda)}{R(\lambda)} \quad (1)$$

where:

$T(\lambda)$ is relative transmission at wavelength λ nm
$S(\lambda)$ is intensity of sample at wavelength λ nm
$R(\lambda)$ is intensity of Teflon reference at wavelength λ nm

It should be noted that the term “transmission spectra” used throughout this text refers to the relative transmission spectra.

2.3. Experimental design

Prior to setting the eggs into the incubator, eggs were preheated for 16 h (first 6 h at 28 °C and the remaining 10 h at 30 °C) to bring the eggs to a uniform temperature when they were placed into the incubator. Just prior to incubation (referred to as “incubation time 0”), the transmission spectra of all eggs were measured. Upon completion of measurements, the eggs were immediately placed in a commercial incubator (SSH-02, Showa Furanki, Saitama, Japan) to incubate at 37.8 °C and 55% of relative humidity as per Lourens et al. (2005). During incubation eggs were turned automatically through an angle of 90° every hour. Between incubation time 1–144 h, eggs were taken out from the incubator every 24 h to measure the spectral transmission of each egg. To minimize the exposure time of

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