

Detection of fertility and early development of hatching eggs with hyperspectral imaging

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Keywords: egg fertility; embryo development; hyperspectral imaging; hatching eggs

Abstract

Detection of fertility prior to incubation or recognition of absence of development during the first days of incubation could allow hatcheries to cull infertile or problem eggs, reducing incubator space, utility costs, and contamination from exploder eggs. This study was conducted to determine the feasibility of using a hyperspectral imaging system to detect changes in eggs related to fertility and development for both white shell eggs and brown eggs. In Experiment 1, 48 single comb white leghorn (SCWL) white shell eggs were obtained unincubated from a commercial hatchery in each of two replicate trials (n=96). On days 0, 1, 2, and 3 of incubation, 12 eggs were imaged using a hyperspectral camera system (wavelengths detected from 400-900nm) and a tungsten-halogen backlight. Each day the eggs were broken out for visual confirmation after imaging. Exposure time was 30 ms, and a ratio of transmittance images at wavelengths 576 and 655 nm was used to detect fertility and embryo development. On Day 3, 23 of 24 eggs were determined fertile at breakout; the imaging system accurately identified the one infertile and 21 of 23 fertile eggs. One egg was determined fertile at Day 0, none at Day 1, and 12 at Day 2. One infertile egg was incorrectly identified as fertile on Day 2. In Experiment 2, in each of two replicate trials 12 brown shell broiler chicken eggs were obtained unincubated, and imaged on days 0, 1, 2, and 3 (same eggs kept and imaged throughout each trial; n=24). Embryo development was then visually confirmed on Day 6. All eggs used were fertile, so the earliest point of embryo development was determined. Exposure time was increased to 250 ms and a transmission ratio of wavelengths used at 576 nm, and a range of wavelengths (682 +/- 13 nm, depending on the light transmission per egg). Detection rates by Day were: Day 1, 13/24 (54%); Day 2 14/24 (58%); and, Day 3, 20/24 (83%). Overall, the hyperspectral imaging system appears capable of detecting developing eggs on Day 3 of incubation at a 91% rate for white shell eggs, and an 83% rate for brown shell eggs.

INTRODUCTION

Hatcheries, like most segments of the poultry industry, are being managed to do more work (set and hatch more eggs) at lower costs and with less labour. Yet two major problems affect the industry: A number of the eggs set take up space and will never hatch, and some of these may cause contamination of the hatching cabinet. Fertility rates for hatching eggs range from 82% to 99%, therefore commercial hatcheries are incubating approximately one billion eggs per year that are infertile. Also, hatcheries load setters (incubators) to maximum capacity to obtain maximum utility efficiency, which may drop hatchability by 0.5% to 2% due to decreased ventilation and higher temperatures in the racks (French, 1997). Elevated temperature control is especially important since the setter thermometer may read lower than the actual temperature in the racks (Mauldin and Buhr, 1995). Currently, about 5% of eggs are candled after 10 days of incubation and "clear" eggs from this group are removed. This sample is used to estimate fertility for farm flocks. All of the infertile eggs included in the 95% of eggs not candled remain in the setters. These remaining infertile eggs are more likely to harbour and grow bacteria or moulds that may cross-contaminate other eggs, especially if they are "exploders".

Hyperspectral imaging has been used for various purposes, including mapping (to analyze conditions on the earth's surface) and medical technology (Dustan, *et al.*, 2000; Shah, *et al.*, 2003). This technology has also been applied to agricultural applications, including determining seed quality, apple defects, and tomato ripeness (Modiano and Deppermann, 2003; Kim *et al.*, 2001; Polder, *et al.*,

2002). For poultry, hyperspectral imaging has been used to detect ingesta and faecal contamination of broiler carcasses (Park *et al.*, 2002; Windham, *et al.*, 2003). Overall poultry carcass quality has also been evaluated (Chao *et al.*, 2003).

Other imaging systems have been developed and tested to address a range of egg problems. One system detected blood spots/bloody whites in shell eggs (Schouenberg, 2003). Another system was developed to detect infertile, dead, or contaminated hatching eggs during transfer at 18 days to minimize cross-contamination during vaccination (Chalker, 2003). Liu *et al.* (2004) reported that chicken egg fertility could be detected using a spectrophotometer to detect the amount of light transmission. There was no description of if or how long the eggs had been incubated. Other research reports indicating that hyperspectral imaging had been used to detect early fertility and development in hatching eggs were not found.

Reducing egg crowding in setters would result from removal of infertile or non-developing eggs, and this could improve hatchability. Removal of eggs likely to become exploders could also improve hatchability and overall eventual flock health. Hyperspectral imaging is a viable technology used in several applications, including agriculture. Therefore, the purpose of this study was to determine the feasibility of using hyperspectral imaging (a rapid, non-invasive technology) to detect infertile or non-developing hatching eggs prior to or during early incubation.

Materials and methods

EXPERIMENT ONE

In each of two replicate trials, 48 Single-Comb White Leghorn (SCWL) eggs (white shell) were obtained from a commercial hatchery prior to incubation (n=96). Eggs were incubated at the University of Georgia research farm hatchery. Incubator conditions were 37.5 C (99.5 F), 85% relative humidity, with hourly automatic turning. Prior to incubation (Day 0) and at Days 1, 2, and 3, 12 eggs were removed from the incubator, imaged, and broken out for visual confirmation of fertility and development. Images were taken with the egg lying on its side, or horizontal axis.

EXPERIMENT TWO

In each of two replicate trials, 12 broiler eggs (brown shell) were obtained from the University of Georgia research farm (n=24). Eggs were incubated at the Russell Research Center in an incubator set at 37.5 C, 85% relative humidity, and automatic hourly turning. For each trial on Days 0 (pre-incubation), 1, 2, and 3 of incubation, the same 12 eggs were removed from the incubator and imaged, then placed back into the incubator and imaged again on subsequent days. At Day 6 the eggs were assessed visually for embryo development. Images were taken with the egg in an upright position with the air cell up, or on its vertical axis.

IMAGING

The imaging system consisted of a 12 bit digital camera with silicon CCD detector connected to a spectrograph and lens assembly (Figure 1), along with a computer and software capable of capturing a hypercube image, with each pixel containing spectra from approximately 400 to 900 nm (Photon Industries, Stennis Space Center, MS, 39529, USA).

Individual eggs were placed on a flat surface with a 29 mm diameter cut-out, on which the egg rested; a tungsten-halogen (150 watt) light mounted below the surface provided a candling effect. SCWL white shell eggs were exposed for 30 ms and a ratio of transmission images at two wavelengths, 576 and 655 nm (derived from the hypercube data as likely indicators of fertility or development). The ratio was used to differentiate pixels from the entire image as positive or negative fertility or embryo development, using an algorithm to mask the negative pixels in the image interior and non-essential background pixels exterior to the egg image (see Figure 2). The same process was used for broiler chicken brown shell eggs, except they were exposed for 250 ms and wavelengths used were 576 nm, then a range of wavelengths (682 $\pm$ 13nm, varying across eggs). Using a range was necessary as the brown shell pigments resulted both in decreased light transmission and increased the amount of variability between individual eggs. The lower wavelength (576 nm) was chosen as it provided the maximum spectral difference between infertile and fertile eggs in Experiment 1. The upper wavelength was chosen (655 nm for Experiment 1, 682 nm plus or minus 13nm for

Experiment 2) as it represented the maximum light transmission through the egg (peak signal to the detector).

Results

EXPERIMENT ONE

Results of the hyperspectral imaging system's ability to detect fertile and developing embryos on days 0 through 3 of incubation are shown in Table 1. Of 96 eggs, 89 were confirmed fertile and 7 infertile through breakout and visual observation. At the system settings and wavelength ratio selected for this study, the system only detected 1 of the 46 fertile eggs on Days 0 and 1. 60% of the fertile and developing eggs were accurately detected on Day 2, and 91% were detected on Day 3. The system inaccurately detected one of the 7 infertile eggs as fertile on Day 2.

EXPERIMENT TWO

All 24 eggs used in the experiment were found to be fertile, so the goal of this experiment was to determine the earliest point at which embryo development could be detected with the hyperspectral imaging system. Results are shown in Table 2. On Day 1, 13 eggs were detected by the imaging system as developing, which improved slightly on Day 2 to detection of 14 eggs. On Day 3, 20 of 24 eggs were detected as developing.

Discussion

For SCWL (white) eggs the imaging system appears to have the capability of detecting fertile and developing eggs with 91% accuracy at Day 3 of incubation, although one false positive result was evident. Brown shell (broiler chicken) eggs were detected by the imaging system as developing at 83% accuracy on Day 3 of incubation.

Selection of the 576 nm wavelength in this study as one of the two important wavelengths was probably due to the close relationship between this wavelength and the 577nm wavelength Schouenberg (2003) detected when blood was found in eggs. Therefore this system as configured is likely detecting the early formation of red blood cells, capillaries, and the blood ring structure associated with early embryo development. The difficulty in specifying a specific upper wavelength for brown shell eggs was due to the lowered light transmission passing through the egg. Other researchers have also reported reduced light transmission through brown shell eggs (Liu *et al.*, 2004; Shafey *et al.*, 2004). Liu *et al.* (2004) combined wavelengths into 20-nm wide bandwidths with average transmittance calculated for that bandwidth. A similar method was used in this study for the upper wavelength for brown shell eggs. A range of wavelengths from 668 to 695 nm (27-nm wide) was used because the light transmission through each individual egg varied, probably due to variance in shell pigmentation.

Further research and data analysis will be required to improve the detection accuracy for either white or brown shell egg embryo development during early incubation using hyperspectral imaging. Because all brown shell eggs were fertile, and because of the high variability of light transmission between individual eggs, data from Experiment 2 should be considered preliminary. A different approach must be developed to detect fertility prior to incubation, as this study apparently detected only the early circulatory system development that occurs with incubation.

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Table 1 Number of Single Comb White Leghorn chicken eggs (white shell) confirmed fertile by breakout, detected fertile/developing by imaging system, and % detection by imaging system on Days 0-3 of incubation (24 eggs per day, 96 eggs total).

<u>Day</u>	<u># Eggs</u>	<u># Confirmed fertile (breakout)</u>	<u># Detected (imaging)</u>	<u>% Detection</u>
0	24	23	1	4
1	24	23	0	0
2	24	20	12	60
3	24	23	21	91

Table 2 Number of broiler chicken eggs (brown shell) confirmed as developing visually, detected as developing by imaging system, and % detection by imaging on Days 1-3 of incubation (same eggs used throughout study, 24 eggs total).

<u>Day</u>	<u># Eggs</u>	<u># Confirmed Developing (visual)</u>	<u># Detected (imaging)</u>	<u>% Detection</u>
1	24	24	13	54
2	24	24	14	58
3	24	24	20	83

Figure 1 Diagram of hyperspectral imaging system, candling stand, and light source (not to scale) for broiler chicken brown shell egg imaging trials. Similar configuration used for SCWL white shell eggs except that eggs were lying horizontally on side instead of upright.

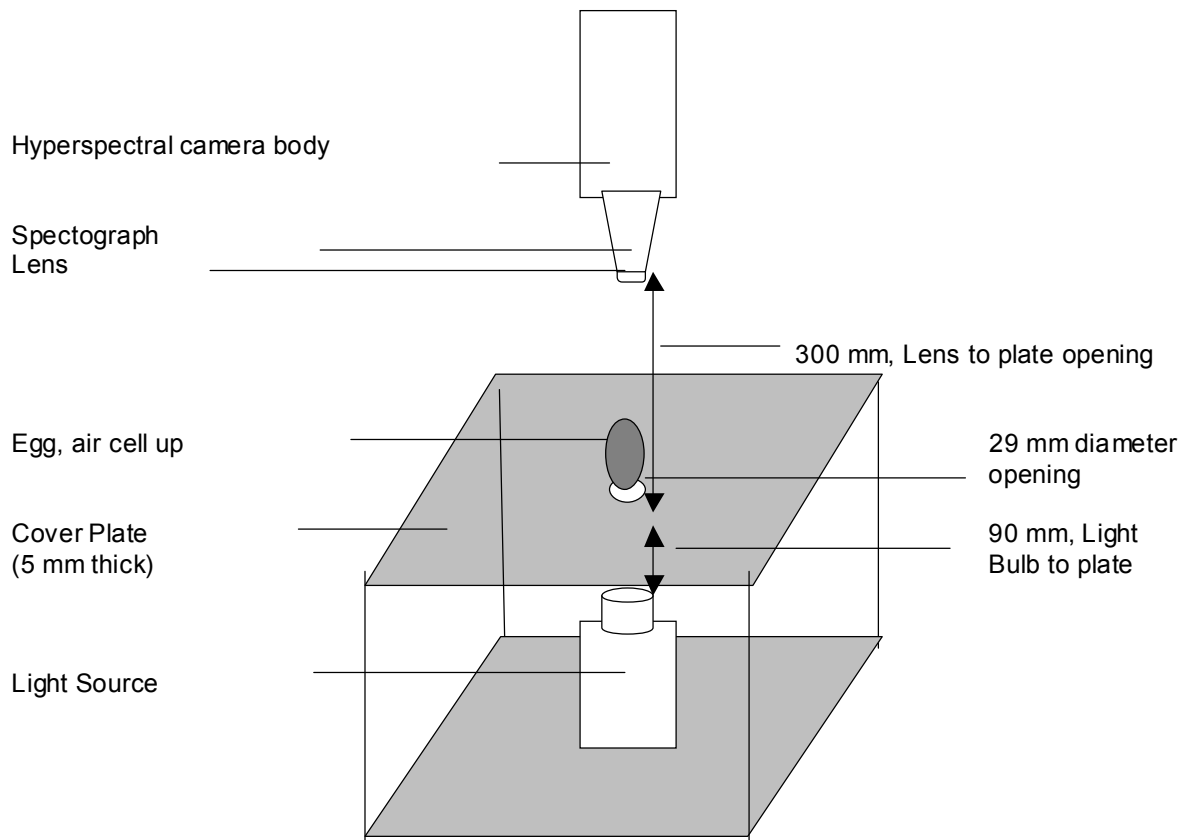
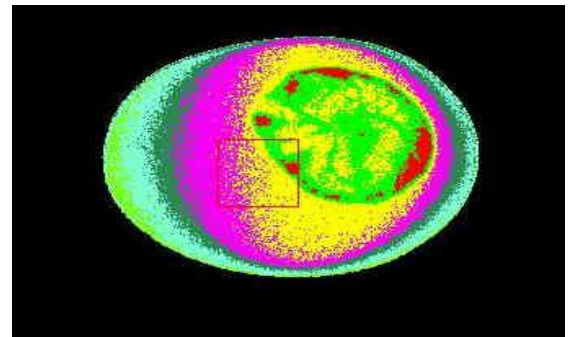


Figure 2 Example of egg development as detected by the hyperspectral imaging system. Side view of a Single Comb White Leghorn (white shell) egg from CCD image to processed spectral image to specific wavelength ratios to final masked image.

Color CCD image of egg at Day 3 of incubation. Box added to show relative position of egg and internal image throughout process



Image composed of multiple wavelengths with colors arbitrarily added for visual differentiation



Ratio of two wavelengths (576 and 655 nm) highlighting early embryo development (color added)

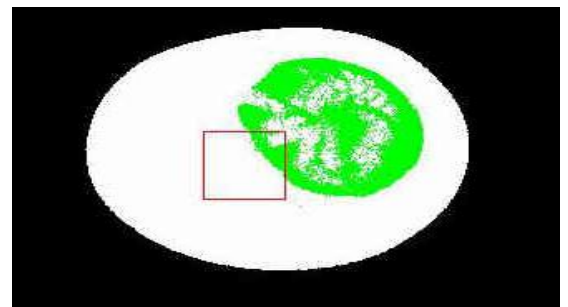


Image with background and non-essential pixels masked

