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Noninvasive Preincubation Sex Determination and Monitoring of Sex-Specific Early Embryonic Growth Rate in Chicken Eggs Using Longitudinal Visible Transmission Spectroscopy
(長軸方向の可視透過分光法を用いたインキュベーション前の非侵襲雌雄判定と性特異的な初期鶏胚成長率のモニタリング)

ABSTRACT

Chicken meat and eggs are a premium source of quality protein and essential nutrients, badly needed by millions of people living in poverty worldwide to achieve UN sustainable development goals (specifically SDGs 2 and 3; i.e., “zero hunger”, and “good health and well-being”). Sex-separated rearing is recommended for broiler operations due to sex-differences in growth rates, feed efficiency, and market demands. While in the layer industry, where the chicks are sexed at hatch, the female chicks (the egg layers) are considered of paramount importance, while the male chicks are culled (7 billion per year) incurring serious costs, waste disposal and animal welfare issues. Thus, it is critical in sex-dependent production of meat and eggs that the sex of chicks is established as early as possible, preferably nondestructively on pre- or early incubation eggs. Hence, to improve the sustainability of poultry production systems, the sorting of eggs based on sex prior to incubation presents many beneficial advantages for both broiler and layer farms in terms of efficient meat production, cost minimization, and humane animal welfare.

Moreover, the precise monitoring of early chick embryonic growth rate (EGR) could aid many potential for research and incubation management applications. From a research point of view, it is of interest to investigate how variations in early embryonic growth rates correlated with late embryonic development right through to pipping and hatching. More importantly, recent studies have demonstrated that avian cells and tissues have an inherent sex identity and that male and female tissues respond differently to the same stimulus. Thus, disregarding sex differences in experiments with chicken embryos can lead to erroneous conclusions, a lack of reproducibility, and wasted effort. Therefore, being able to monitor sex-specific early embryonic growth rate in a nondestructive way could potentially reveal useful information that could provide linkages between pre- and early incubation egg characteristics, hatching time, and post-hatch chick performance (e.g., chick weight).

Previous research observations have pointed to the possibility of male-biased maternal resource (yolk) allocation in broiler eggs, a phenomenon consistent with findings (mechanism of yolk accumulation and sex determination) in wild bird species. These include sex-differences in broiler embryo size during incubation, as well as after hatching and the correlation of larger yolk mass of broiler eggs to the larger body mass of those embryos. This suggests that egg components (yolk) associated with sex-biased allocation could be most prevalent and detectable at the laying of the egg prior to incubation and that this could act as a fingerprint for preincubation sex determination. Besides, the production of blood (erythropoiesis) is known to take place from day 2 of incubation in a fertile egg. Given that erythropoiesis can be equated with early embryonic growth rate, it was hypothesized that blood pigment hemoglobin (having three absorption bands at 415, 539, and 575 nm) could act as a specific spectral fingerprint for changes in embryo growth rate. However, for brown colored eggshells containing protoporphyrin, there is a confounding factor of three absorption bands at 539, 589 and 643 nm, while the eggshell itself absorbs most of the light below 500 nm. This, together with variability in egg characteristics such as eggshell thickness and yolk color, has led researchers to combine reference wavelengths to minimize interference (e.g. at 598 and 610 nm) with transmittance measurements at 575 nm to detect blood in both white and brown Table eggs. The overall objective of this research was to develop nondestructive, sensitive, and consistent techniques for preincubation sex determination of broiler eggs and subsequent monitoring of sex-specific early EGR and its correlations with chick

performance (hatching time and chick weight) using longitudinal visible transmission spectroscopy combined with multivariate analysis.

To achieve preincubation sex determination, we measured the transmission spectra of chicken broiler eggs prior to incubation. After that, we identified the most sensitive region of spectral absorbance in the range from 500 to 950 nm associated with differences between male and female eggs using feature selection techniques, followed by building and evaluating a sex prediction model based on these selected absorbance features. Consecutively, the potential contribution of yolk ratio (yolk weight/ whole egg weight) to these sex-related absorbance differences in this selected spectral region were also examined. Secondly, we measured transmission spectra from day 0 to 9 of incubation for monitoring sex-specific early embryo growth rate in both white layer and light brown broiler eggs, the ratio of transmission intensities at 575 nm with a reference wavelength (598 nm for white eggs and 610 nm for brown eggs; blood value) from the longitudinal visible light transmission spectrum were used.

A logistic regression classifier combined with selected features (t-test method) achieved an overall accuracy of 76% for determining preincubation male and female broiler eggs. Further, the contribution of the yolk ratio to this sex classification was confirmed. Secondly, embryonic development was detected from day 3 of incubation in both white layer and light brown broiler eggs, respectively. At day 3, sex-differences ($P<0.001$) in blood values were found only in white layer eggs, while differences in embryonic blood production rates and spectral masking of the eggshell pigmentation masked these sex-differences ($P>0.05$) in brown broiler eggs. Besides, on day 7, female embryos of both layer and broiler eggs had significantly lower ($P<0.05$) mean blood values than males; possibly due to estrogen synthesis receptors and enzymes promoting erythropoiesis (blood production) in female embryos prior to this. In addition, the hatching time of male chicks had a higher correlation (Correlation value=0.36, $P<0.01$) with the blood value measured on day 4 than females (correlation value=0.21), presumably due to higher blood levels in males at this time. Moreover, the blood value on day 6 had a significant ($P<0.0001$) correlation with the weight of newly hatched chicks (correlation value=0.54).

In conclusion, this research could enable sustainable sex-specific broiler meat production and advance precision incubation management, which will contribute to future food security and healthy human lives (i.e., SDGs goals 2 and 3). Moreover, this research could also open many new dimensions of nondestructive research in precision poultry production (sex determination during early incubation) and biomedical engineering fields involving angiogenesis.