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Short Communication

Detection of embryo mortality and hatch using thermal differences among incubated chicken eggs

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ABSTRACT

Accurate diagnosis of both the stage of embryonic mortality and the hatch process in incubated eggs is a fundamental component in troubleshooting and hatchery management. However, traditional methods disturb incubation, destroy egg samples, risk contamination, are time and labour-intensive and require specialist knowledge and training. Therefore, a new method to accurately detect embryonic mortality and hatching time would be of significant interest for the poultry industry if it could be done quickly, cheaply and be fully integrated into the process. In this study we have continuously measured individual eggshell temperatures and the corresponding micro-environmental air temperatures throughout the 21 days of incubation using standard low-cost temperature sensors. Moreover, we have quantified the thermal interaction between eggs and air by calculating thermal profile changes (temperature drop time, drop length and drop magnitude) that allowed us to detect four categories of egg status (infertile/early death, middle death, late death and hatch) during incubation. A decision tree induction classification model accurately (93.3%) predicted the status of 105 sampled eggs in comparison to the classical hatch residue breakout analyses. With this study we have provided a major contribution to the optimisation of incubation processes by introducing an alternative method for the currently practiced hatch residue breakout analyses.

1. Introduction

Hatchability is key for assessing incubation results. Thus, investigating hatching failures is an increasingly recognised concern for the modern poultry industry in attempting to uncover the basis of egg fertility and embryonic mortality (Sellier et al., 2006). Besides, Non-viable eggs also take up space that can be used for fertile eggs and a potential source of bacteria and/or fungi and can thus cause contamination. During incubation, egg candling is normally carried out in the middle of the process (day 10) or during transfer (day 18), in order to identify infertile eggs and mortality. However, egg breakout analyses requires invasive intervention, destroys egg samples, kills embryos, risks contamination, is time and labour-intensive and requires specialist knowledge and training (Sellier et al., 2006; Liu and Ngadi, 2013). Detection of infertility and mortality is not the only issue, investigation of the hatch evolution is also very important to evaluate uniformity of the batch. In practice, the moment of hatch is examined by taking several hatchery baskets out of the incubator and checking the number of chicks hatched. However, this procedure may require the door of the incubator to be opened and is carried out several times during the process which may significantly affect the incubation conditions and interrupt the hatching process (Tong et al., 2015). Therefore, there is an increased interest in an alternative and less invasive method for the detection of egg fertility and monitoring of embryo mortality and hatch. This paper attempts to show the benefit of using eggshell temperature sensors during the whole incubation to quantify thermal profile differences among infertile eggs, eggs containing dead embryos at different developmental stages (early, middle and late) and eggs that succeed in hatching.

2. Material and methods

Ross 308 eggs (Henry Stewart & Co. Ltd., Lincolnshire, United Kingdom) were incubated and hatched in a custom small-scale

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incubator (Petersime NV., Zulte, Belgium) using a standard 21-day incubation programme. Twenty out of six hundred eggs were randomly picked and individually labelled as focal eggs in each incubation trial to serve as samples for the current study. In total, 120 focal eggs from six repetitions were analysed. Standard low-cost contact temperature sensors (Romanini et al., 2013) were attached to the equator of the focal egg eggshells. Another temperature sensor was positioned 1 cm away from each focal egg to record the corresponding micro-environmental air temperature (T_{air}). The eggshell temperatures (T_{egg}) of each focal egg and T_{air} were recorded every minute throughout the entire incubation.

At the end of incubation, hatch residues were evaluated by an expert in breakout analysis (Petersime NV, Zulte, Bekgium). Egg status was determined according to the developmental stage of the dead embryo (Hamburger and Hamilton, 1992) and allocated into the following categories: infertile (INF), early death (ED), middle death (MD) and late death (LD). Cracked and contaminated eggs were excluded from the analyses. This study had an ethical approval from the Royal Veterinary College Ethics and Welfare Committee.

Both T_{egg} and T_{air} time series data were processed in Matlab® (The Math Works, Inc., Natick, United States) using built-in codes and functions of the Captain Toolbox (Taylor et al., 2007).

The temperature difference (ΔT) between T_{egg} and T_{air} was calculated and filtered to produce the $\Delta T_{filtered}$ signal representing the final thermal profile. The $\Delta T_{filtered}$ signal was further processed using a 10-h average window approach to investigate differences in temperature. The following parameters of an identified temperature drop in $\Delta T_{filtered}$ were quantified for the MD, LD and focal eggs (Fig. 1): (1) the time when the lowest drop occurred (drop time); (2) the duration of the drop from a maximum local value to the minimum local value (drop length); and (3) the temperature scale of the drop (drop magnitude).

Statistics were performed using the statistical software package Minitab® (Minitab Inc., State College, United States). Initially, the thermal profiles of focal eggs were grouped into one category of egg status (INF, ED, MD, LD or *H*) according to the results of the hatch residue breakout analyses. The Anderson–Darling (Anderson and Darling, 1954) and the Bartlett's tests (Ridgman, 1990) were used to test normality and homogeneity of variances, respectively. The parameters extracted from $\Delta T_{filtered}$ were summarised using descriptive statistics (mean followed by the standard error of the mean). A single ANOVA followed by post hoc test, with a significance level of 0.05, was used to test the differences in drop length and drop magnitude among the egg categories.

The data set was classified using a decision tree induction model (Quinlan, 1986). Thermal profile derived parameters (temperature drop time, drop length and drop magnitude) were inputs towards the classification of the egg status (INF, ED, MD, LD or *H*) as output. The application WEKA 3.6.9 (University of Waikato, New Zealand) was used to develop a J48 decision-based classifier (Hall et al., 2009) with a 10-fold cross validation approach. Egg status classified by the decision tree model, was compared to the reference status from breakout analyses. The classification performance was expressed in terms of binary classification statistics (Olson and Delen, 2008): TP rate (rate of true positives); FP rate (rate of false positives); and ROC-curve (the ability of performing correctly classification).

3. Results

The 105 focal eggs were grouped according to the results of the hatch residue breakout analysis, as following: INF ($n=15$), ED ($n=3$), MD ($n=12$), LD ($n=11$) and *H* ($n=64$). The thermal profile interactions between T_{egg} and T_{air}, throughout the entire incubation time (512 h), of two focal eggs from each egg status category are illustrated, as examples, in Fig. 2.

A common feature (notable temperature drops on Fig. 2C, D and E) was found for all focal eggs categorized into MD, LD and *H*. Eggs in the status category INF/ED did not show the same pattern (Fig. 2A and B). Those temperature drops were associated to the time of embryonic mortality in the cases of MD and LD, or to the time which chicks emerge from their shells in the case of *H* eggs. Fig. 3 shows the normal distribution curves of the temperature drop time for the MD, LD and *H* eggs. This result shows overlap between the temperature drop time registered for the egg categories MD and LD or, most clearly for LD and *H*.

In addition, quantitative differences were identified in the thermal profiles (temperature drop length and drop magnitude) of MD, LD and *H* eggs (Table 1). The temperature drop length found in the *H* eggs (mean of 6.78 h) was significantly lower than LD (15.82 h) and MD (18.75 h) ($P<0.05$). Furthermore, differences in drop magnitude were found among the egg status categories. The highest temperature drop was obtained for the *H* eggs with a mean value of 0.73 °C ($P<0.01$). MD and LD eggs showed smaller averaged drop magnitudes of 0.19 °C and 0.30 °C, respectively.

Fig. 4 shows the results of a binary decision tree and the thresholds used for classification into one of the four outcome egg status (INF/ED, MD, LD and *H*) according to the thermal profiles of the interaction between T_{egg} and T_{air}. At the top level of the decision tree there is the root node, at which the classification begins. It tests all focal eggs for temperature drop time $<=455$ h of incubation. Instances that satisfy this condition are passed down

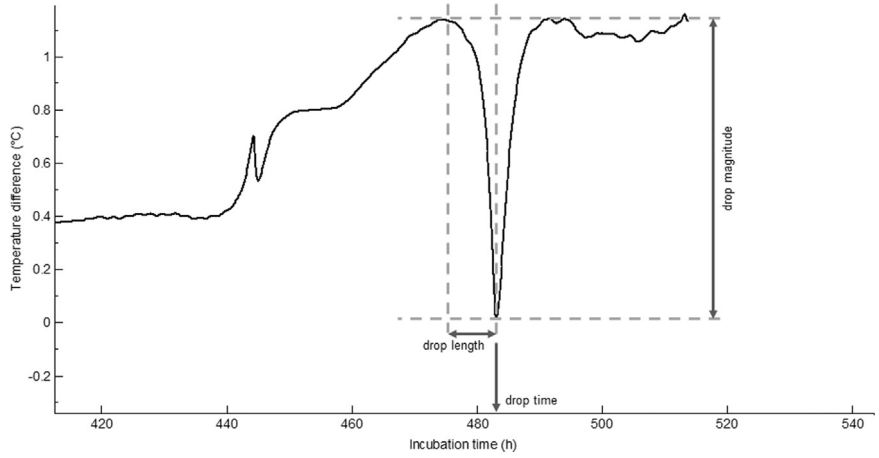

Fig. 1. An example of the quantitative characteristic (drop time, drop length and drop magnitude) of the $\Delta T_{filtered}$ time series data.