

This is the peer-reviewed, manuscript version of the following article:

Q. Tong, C.E.B. Romanini, V. Exadaktylos, I.M. McGonnell, D. Berckmans, C. Bahr, H. Bergoug, N. Roulston, M. Guinebretière, N. Eterradossi, R. Verhelst, T.G.M. Demmers (2016). Detection of embryo mortality and hatch using thermal differences among incubated chicken eggs. *Livestock Science* 183, Jan 2016, pp. 19-23.

The final version is available online via <http://dx.doi.org/10.1016/j.livsci.2015.11.004>.

© 2016. This manuscript version is made available under the CC-BY-NC-ND 4.0 license <http://creativecommons.org/licenses/by-nc-nd/4.0/>.

The full details of the published version of the article are as follows:

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

AUTHORS: Q. Tong, C.E.B. Romanini, V. Exadaktylos, I.M. McGonnell, D. Berckmans, C. Bahr, H. Bergoug, N. Roulston, M. Guinebretière, N. Eterradossi, R. Verhelst, T.G.M. Demmers

JOURNAL TITLE: Livestock Science

VOLUME/EDITION: 183

PUBLISHER: Elsevier

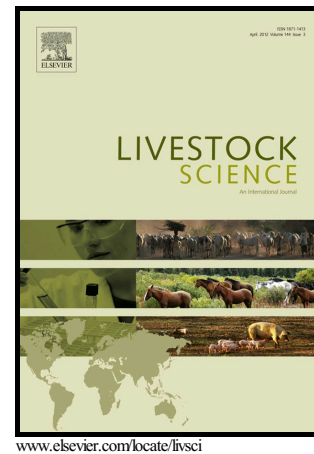
PUBLICATION DATE: 6 November 2015 (online)

DOI: 10.1016/j.livsci.2015.11.004

# Author's Accepted Manuscript

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

Q. Tong, C.E.B. Romanini, V. Exadaktylos, I.M. McGonnell, D. Berckmans, C. Bahr, H. Bergoug, N. Roulston, M. Guinebrière, N. Etteradossi, R. Verhelst, T.G.M. Demmers



PII: S1871-1413(15)30030-5  
DOI: <http://dx.doi.org/10.1016/j.livsci.2015.11.004>  
Reference: LIVSCI2879

To appear in: *Livestock Science*

Received date: 13 March 2015  
Revised date: 4 November 2015  
Accepted date: 5 November 2015

Cite this article as: Q. Tong, C.E.B. Romanini, V. Exadaktylos, I.M. McGonnell, D. Berckmans, C. Bahr, H. Bergoug, N. Roulston, M. Guinebrière, N. Etteradossi, R. Verhelst and T.G.M. Demmers, Detection of embryo mortality and hatch using thermal differences among incubated chicken eggs, *Livestock Science*, <http://dx.doi.org/10.1016/j.livsci.2015.11.004>

This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and a review of the resulting galley proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain

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

Q. Tong<sup>a\*</sup>, C. E. B. Romanini<sup>b\*</sup>, V. Exadaktylos<sup>b</sup>, I. M. McGonnell<sup>a</sup>, D. Berckmans<sup>b</sup>, C. Bahr<sup>b</sup>,  
H. Bergoug<sup>c</sup>, N. Roulston<sup>d</sup>, M. Guinebretière<sup>c</sup>, N. Eterradossi<sup>c</sup>, R. Verhelst<sup>d</sup>, T. G. M. Demmers<sup>a</sup>

<sup>a</sup> The Royal Veterinary College, Hawkshead Lane, North Mymms, Hatfield, AL9 7TA  
Hertfordshire, United Kingdom

<sup>b</sup> Division M3-BIORES: Measure, Model & Manage Bioresponses, KU Leuven, Kasteelpark  
Arenberg 30 - box 2456, B-3001 Leuven, Belgium

<sup>c</sup> UEB-Anses, Ploufragan-Plouzané Laboratory, Avian and Rabbit: Epidemiology and Welfare  
Unit / Virology-Immunology-Parasitology Unit, BP 53 Ploufragan, 22440 France

<sup>d</sup> Research and Development, Petersime N.V., Centrumstraat 125, B-9870 Zulte (Olsene),  
Belgium

\* These authors have contributed equally to this study

Corresponding author: Theo Demmers. E-mail: tdemmers@rvc.ac.uk; phone: +44 (0)1707  
666945; Royal Veterinary College, Hawkshead Lane, North Mymms, Hatfield, AL9 7TA  
Hertfordshire, United Kingdom

**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 optimization of incubation processes by introducing an alternative method for the currently practiced hatch residue breakout analyses.

**Keywords:** egg breakout, eggshell temperature, embryo status, hatching time.

## **Introduction**

Hatchability is key for assessing incubation results. Thus, investigating hatching failures is an increasingly recognized 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.

### **Material and methods**

Ross 308 eggs (Henry Stewart & Co. Ltd., Lincolnshire, United Kingdom) were incubated and hatched in a custom small-scale incubator (Petersime NV., Zulte, Belgium) using a standard 21-day incubation program. 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 ( $T_{\text{air}}$ ). The eggshell temperatures ( $T_{\text{egg}}$ ) of each focal egg and  $T_{\text{air}}$  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  $T_{\text{egg}}$  and  $T_{\text{air}}$  time series data were processed in Matlab<sup>®</sup> (The Math Works, Inc., Natick, United States) using built-in codes and functions of the Captain Toolbox (Taylor et al., 2007). The temperature difference ( $\Delta T$ ) between  $T_{\text{egg}}$  and  $T_{\text{air}}$  was calculated and filtered to produce the  $\Delta T_{\text{filtered}}$  signal representing the final thermal profile. The  $\Delta T_{\text{filtered}}$  signal was further processed using a 10-hours average window approach to investigate differences in temperature. The following parameters of an identified temperature drop in  $\Delta T_{\text{filtered}}$  were quantified for the MD, LD and H focal eggs (Figure 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).

### **Insert Figure 1**

Statistics were performed using the statistical software package Minitab<sup>®</sup> (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  $\Delta T_{\text{filtered}}$  were summarized 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).

## 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  $T_{\text{egg}}$  and  $T_{\text{air}}$ , throughout the entire incubation time (512 hours), of two focal eggs from each egg status category are illustrated, as examples, in Figure 2.

### Insert Figure 2

A common feature (notable temperature drops on Figures 2C, 2D and 2E) 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 (Figure 2A and 2B). 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. Figure 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.

### Insert Figure 3

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 hours) was significantly lower than LD (15.82 h) and MD (18.75h) ( $P < 0.05$ ). 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.

### **Insert Table 1**

Figure 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  $T_{\text{egg}}$  and  $T_{\text{air}}$ . 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  $\leq 455$  h of incubation. Instances that satisfy this condition are passed down to the left of the tree to the second root node. It corresponds to a new test (temperature drop time  $\leq 0$  h), meaning that there is no such notable temperature drop on the thermal profile ( $\Delta T_{\text{filtered}}$ ). If this is the case, this test passed once more down to the left reaching a leaf node INF/ED and no more tests are needed. Focal eggs are classified as MD if the temperature drop time  $\leq 383$  h, or as LD if the temperature drop time is  $> 383$  h but  $\leq 455$  h of incubation. From the top root node to the right, focal eggs with a temperature drop time  $> 455$  h are further tested at intermediate nodes (temperature drop length and drop magnitude) to be classified into one single egg status category (LD or H).

### **Insert Figure 4**

The performance of the decision tree classification model is shown in Table 2. The overall success rate of the classification model was 93%. Each element in Table 2 is a count of focal eggs. Rows represent the reference egg status categories classified by hatch residue breakout



analyses and columns represent the predicted egg status by the decision tree model. A total of 98 out of 105 focal eggs were correctly classified. The model was 100% precise at classifying INF/ED eggs. 10 out of 12 MD eggs were correctly classified and 2 were incorrectly categorized as LD. The highest error rate was in the LD eggs with 3 errors in 11 focal eggs due to the limited sample size. The classification model correctly classified 62 out of 64 H eggs. Detailed accuracy results for each status category are shown in Table 3. A ROC-curve value of 1 represents a perfect classifier while values approaching 0.5 indicate a classifier with reduced ability, comparable to random guessing. ROC-curve values were higher than 0.9 for each egg status category.

**Insert Table 2**

**Insert Table 3**

### **Discussion**

The quantification of the changes in the thermal profile interaction between  $T_{\text{egg}}$  and  $T_{\text{air}}$  for the whole incubation period allowed us to identify four categories of egg (fertility/early death, middle death, late death and hatch). As expected, the decision tree classification model indicated ‘drop time’ as the criteria holding the greatest information. We cannot distinguish between an infertile egg and an egg with early embryonic mortality because they showed similar thermal profiles throughout incubation. Furthermore, drop time alone seems unlikely to be enough to distinguish classes of embryo status in the overlapping regions when our results are extrapolated to a population level. Temperature drops were identified when embryos died or hatch as a response to an abrupt change in embryonic heat production. This allowed us to precisely determine the time for embryonic mortality and hatch. The quickest and largest temperature drop was observed for hatched eggs. After external pipping, the chick emerges from the egg and

causes huge amount of heat release due to evaporation of the fluid left in the internal eggshell membrane by the recently hatched chick (Romanini, et al., 2015). Therefore, the temperature drop caused by hatch was different from the temperature drop caused by embryo death in terms of drop length and drop magnitude. We have developed a classification model with a small number of misclassification (7 errors out of 105 focal eggs), corresponding to an overall 93.3% accuracy. With the increase of egg samples, especially for late dead eggs, the classification model could be fine-tuned and yield even higher accuracy.

### **Conclusion**

A method was proposed in this study to determine the egg status using the temperature sensors based on the different thermal profile changes throughout the incubation period. Accurate detection of embryo status and the moment of hatch in commercial hatcheries would be of significant interest to the poultry industry if it can be done quickly and cheaply. The results presented herein correspond to the absolute first step, distinction of thermal profiles of eggs from different status, towards the development of future automated monitoring systems for incubated chicken eggs.

### **Acknowledgement**

This study is part of the EU Marie-Curie Initial Training Network, BioBusiness project (FP7-PEOPLE-ITN-2008) and CNPq - Brazil, which the authors are grateful for the financial support.

### **References**

- Anderson, T.W., Darling, D.A., 1954. A test of goodness of fit. *J. Am. Stat Assoc.* 49, 765-769.
- Bakst, M.R., Akuffo, V., 2002. Impact of egg storage on embryo development. *Avian Poult. Biol. Rev.* 13, 125-131.

- Hall, M., Frank, E., Holmes, G., Pfahringer, B., Reutemann, P., Witten, I.H., 2009. The WEKA data mining software: an update. *ACM SIGKDD Explor. Newsl.* 11, 10-18.
- Hamburger, V., Hamilton, N.L., 1992. A series of normal stages in the development of the chick embryo. *Dev. Dynam.* 195, 231-272.
- Liu, L., Ngadi, M.O., 2013. Detecting fertility and early embryo development of chicken eggs using near-infrared hyperspectral imaging. *Food Bioprocess Tech.* 6, 2503-2513.
- Olson, D. L., Delen, D., 2008. *Advanced data mining techniques*, first ed. Springer.
- Quinlan, J.R., 1986. Induction of decision trees. *Mach. Learn.* 1, 81-106.
- Ridgman, W.J., 1990. Review of (Snedecor GW and Cochran G 1989) statistical methods, 8th edition. *J. Agric. Sci.* 115, 153-153.
- Romanini, C.E., Exadaktylos, V., Tong, Q., McGonnell, I., Demmers, T.G., Bergoug, H., Eterradossi, N., Roulston, N., Garain, P., Bahr, C., Berckmans, D., 2013. Monitoring the hatch time of individual chicken embryos. *Poult. Sci.* 92, 303-309.
- Romanini, C.E.B., Exadaktylos, V., Hong, S.W., Tong, Q., McGonnell, I., Demmers, T.G.M., Bergoug, H., Guinebretière, M., Eterradossi, N., Roulston, N., Verhelst, R., Bahr, C., Berckmans, D., 2015. An insight into the heat and mass transfer mechanisms of eggshells hatching broiler chicks and its effects to the hatcher environment. *J. Therm. Biol.* 48, 69-76.
- Sellier, N., Brillard, J.P., Dupuy, V., Bakst, M.R., 2006. Comparative staging of embryo development in chicken, turkey, duck, goose, guinea fowl, and Japanese quail assessed from five hours after fertilization through seventy-two hours of incubation. *J. Appl. Poult. Res.* 15, 219-228.
- Taylor, C.J., Pedregal, D.J., Young, P.C., Tych, W., 2007. Environmental time series analysis and forecasting with the Captain toolbox. *Environ. Modell. Softw.* 22, 797-814.

Tong, Q., Demmers, T., Romanini, C.E., Bergoug, H., Roulston, N., Exadaktylos, V., Bahr, C., Berckmans, D., Guinebretiere, M., Eterradossi, N., Garain, P., McGonnel, I.M., 2015. Physiological status of broiler chicks at pulling time and the relationship to duration of holding period. *Animal*. 9, 1181-1187.

### Figure captions

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

**Figure 2** The corrected  $\Delta T_{\text{filtered}}$  profiles throughout incubation of: A) Two early death eggs; B) Two mid death eggs; C) Two late death eggs; D) Two hatched eggs. The variation between 430-450 hours of incubation was caused by the temperature change during transfer of the eggs from incubation trays to hatching baskets.

**Figure 3** Fitted normal distribution curves for the time of temperature drop (drop time) occurred in  $\Delta T_{\text{filtered}}$  for MD, LD and H embryo status categories in the function of incubation time. MD = middle death (n = 12), LD = late death (n = 11), H = hatched eggs (n = 64).

**Figure 4** J48 decision tree classification model for egg status into the INF/ED, MD, LD and H categories with the specification of the classification rules thresholds. INF/ED = infertile or early death, MD = middle death, LD = late death, H = hatched eggs.

### Table Captions

**Table 1** The comparisons of the drop length and drop magnitude among MD, LD and H focal eggs categories.

Item	Sample size	Drop length (h)	Drop magnitude (°C)
INF/ED	18	/	/
MD	12	$18.75 \pm 1.80^a$	$0.19 \pm 0.02^b$

LD	11	$15.82 \pm 1.90^b$	$0.30 \pm 0.03^b$
H	64	$6.78 \pm 0.14^c$	$0.73 \pm 0.03^a$

INF/ED = infertile or early death, MD = middle death, LD = late death, H = hatched eggs;

<sup>a-c</sup> means within the column with different superscripts differ significantly ( $P < 0.05$ ).

**Table 2** Results of the classification tree in a confusion matrix form using a 10-fold cross-validation.

Reference status from hatch residue analyses	Classified by the decision tree model			
	INF/ED	MD	LD	H
INF/ED (n = 18)	18	0	0	0
MD (n = 12)	0	10	2	0
LD (n = 11)	0	1	8	2
H (n = 64)	0	0	2	62

INF/ED = infertile or early death, MD = middle death, LD = late death, H = hatched eggs, n = sample size of focal eggs.

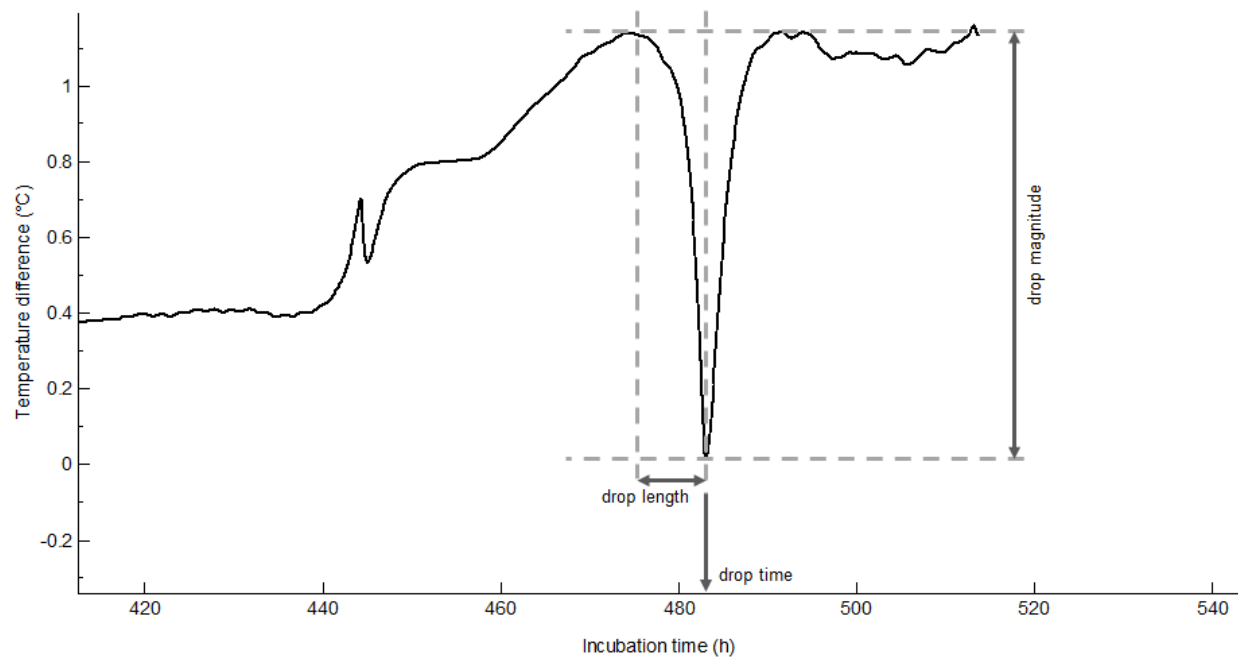
**Table 3** Detailed accuracy results of the decision tree model by category of egg status

Egg status Category	INF/ED	MD	LD	H	Weighted Average
TP rate	1	0.83	0.73	0.97	0.933
FP rate	0	0.01	0.04	0.05	0.035
ROC-curve	1	0.99	0.91	0.99	0.985

INF/ED = infertile or early death, MD = middle death, LD = late death, H = hatched eggs, TP rate = rate of true positives, FP rate = rate of false positives, ROC-curve = classification ability.

### Highlights

- We continuously monitored eggshell temperature throughout the incubation period
- It showed different thermal profiles among four categories of egg status
- Novel method to detect embryonic mortality and hatching time and suitable to automation
- The precise time of embryonic mortality and hatch can be determined
- Classification accuracy was 93% compared to hatch residue breakout analyses



Accepted manu

