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### Shell temperature: how shall we tell if a still gosling is under the eggshell?

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#### Abstract

The present investigation was aimed at predicting a still (i.e., dead) vs. live embryo within a hatching goose egg by measuring the eggshell cooling rate. For this, we daily measured the temperature (T) values on the shell surface of goose eggs after they were removed from the incubator and during further natural cooling. T was recorded every 0.5 hours for further 1.5 hours of cooling. It was possible to recognize eggs with dead embryos using the combination of T, egg weight (W), and surface area (S). The resultant indicator (TS/W) was called *specific temperature index* (STI). The mathematical relationship using STI measurements between Days 8 to 13 facilitated 80% correct identification of the eggs with dead embryos. Additionally, we derived mathematical dependencies for shell weight ( $W_s$ ) and thickness (t) by utilizing the values of  $W_s$  egg volume (V), S, the average T of all measurements taken, as well as the drop in T during 1.5 hours of natural cooling. The key advantage of these parameters was their measurement and/or calculation by applying non-destructive methods. The integrated application of these parameters resulted in achieving high calculation accuracy as judged by correlation coefficients of 0.908 for  $W_s$  and 0.593 for t. These novel mathematical models have the potential to decrease hatching waste by predicting embryo viability. Our research will add to a toolkit for non-invasive egg assessment that is useful in the poultry industry, research on eggs, and engineering.

**Keywords:** Goose eggs; Shell temperature; Egg cooling rate; Specific temperature index; Hatchability; Non-destructive testing

# Nomenclature

d	Air cell diameter in mm
D	Egg density in g·cm <sup>-3</sup>
h	Air cell height in cm
S	Eggshell surface area in cm <sup>2</sup>
STI	Specific temperature index
Т	Temperature on the shell surface in °C
Tav	Average temperature of all measurements taken in °C
$\Delta T_{0.5}$ , $\Delta T_{1.5}$	Drop in shell temperature during 0.5 and 1.5 hours , respectively, at natural cooling of
	the egg in °C/hour
t	Eggshell thickness in cm
V	Egg volume in cm <sup>3</sup>
Vac	Air cell volume in cm <sup>3</sup>
<i>W</i> <sub>1</sub> to <i>W</i> <sub>13</sub>	Egg weights on the respective day of the incubation in g
Ws	Eggshell weight in g
у	Conditional value (1 or 2) of the category into which the examined goose egg falls

#### 1. Introduction

There are three main stages in the process of developing engineering solutions and systems for unraveling a particular research or practical problem. At the first stage, a measuring tool is developed that captures a specific parameter. At the second stage, an actuator is created and implemented for a working process, depending on the readings of a given measuring instrument. The third stage targets the development of computational algorithms that ensure the functionality of the entire system. Herewith, it is not necessary that exactly this order of the above stages be observed for implementing new engineering developments. For example, an incubator is the most typical actuator for the needs of industrial poultry farming, in particular, for its key operation associated with hatching eggs [1]. A given temperature (T) regime that is most optimal for the process of embryonic development [2-4] can be provided by computational algorithms. To date, a large number of engineering solutions have been developed allowing for measuring T of a certain medium and/or surface with varying accuracy. If the eggs are taken out of the incubator and the T change of their cooling is measured using a specific sensor, one can develop an appropriate algorithm that describes the relationship between the cooling indicators and the useful qualitative parameters of the eggs. If such dependencies exist, one can invent an actuator that will allow to sort and cull eggs based on the calculation results using the developed algorithm. Thus, since the suitable T measuring instruments are available, the related functional relationships can be developed as a basis for the next stage of a comprehensive engineering solution for sorting and culling hatching eggs (e.g., [5]).

The example with the temperature regime for egg cooling and its possible relationship with certain useful characteristics were not chosen in vain. Exploration of influences of the T regimes on egg parameters has become the subject of several serious studies. For example, a mathematical model of the process of T and humidity changes in an egg during incubation developed by Meijerhof and van Beek [6] makes it possible to assess the condition and development of the embryo. French [7], as a result of studies of several models of T changes in eggs during incubation, concluded that the data from such modelling are effective for the development of new incubator designs. At the same time, the relationship between the T parameters of an egg and the nuances of its incubation is interesting not only for artificial, but also natural incubation, making it possible to study the behavioral characteristics of wild bird species during the incubation period (e.g., [8]).

However, more work in this direction was aimed at finding a relationship with the possibility of identifying unfertilized eggs and eggs with non-viable embryos. The promise of such studies is that egg T, in addition to the T regime of the incubator, is also influenced by the heat production of the embryo [9]. In this regard, it is likely that there will be some differences in T between eggs of different hatching categories. However, such differences were noted only in the second half of the incubation process [10] or even at the very end, immediately before hatching [11]. Romanini et al. [12], in their studies monitoring hatching of broiler eggs,

found T differences on the shell surface of 2 to 6 °C during the last three incubation days. At the same time, the authors used special T sensors that recorded the egg T every minute. Sunardi et al. [13] developed a system for automatic T monitoring of eggs using a thermal imaging camera and smartphone camera, which indicates the presence of quite effective developments in the implementation of engineering solutions for the process of such measurements.

Some researchers have paid attention not only to differences in *T* on the surface of eggs, but also to differences in the process of *T* changes as a result of their cooling and/or heating. For example, Mortola and Gaonac'h-Lovejoy [14] showed that the cooling time of chicken eggs varied slightly at different stages of incubation.

Thus, it can be concluded that analysis of the shell surface T can be a very effective tool for monitoring eggs during incubation, in particular their possible identification with regard to fertilization and/or viability. Considering that similar studies have not been carried out on goose eggs, we aimed here at evaluating the usability of T shell measurements of goose eggs to assess their incubation quality and morphological data. These studies were carried out in parallel with those whose results we earlier described in Narushin et al. [15].

## 2. Material and methods

#### 2.1. Ethics

The research was in line with the requirements of the European Convention for the Protection of Vertebrate Animals used for Experimental and other Scientific Purposes (ETS No. 123, Strasbourg, 1986). Only manipulating eggs was done for this study; no direct experiments on animals were used.

## 2.2. Experimental design

These studies were performed according to a methodology described in more detail elsewhere [15]. Briefly, the basic information on experimental design is presented below.

Eighty goose eggs were collected from Ballyrichard Farm in Arklow (Ireland; 52°50′5″ N, 6°7′49″ W), Ireland, using the Legarth goose flock. Appropriate flock management and housing criteria were adhered to as previously established [16–18]. The eggs were incubated on site, in the farm's hatchery, in a Bristol S-60 incubator (Bristol, UK) — with temperature and relative humidity set in accord with the manufacturer's instructions and prior experience — that automatically turned eggs through 90° (45° either side from the vertical) at hourly intervals (see [18]). All eggs were weighed (*W*), both before being placed in the incubator and daily during their incubation. Geometric dimensions of eggs were measured according to the methods

described in Narushin et al. [19,20] and used further for computing volume (*V*) and shell surface area (*S*) using the formulae presented in Narushin et al. [21]. Egg density (*D*) was calculated as the ratio of *W* to *V*.

To ensure the greatest sample size of substandard eggs, the experiment was planned for June 2023, at the last phase of the laying season (see [22,23]). Because of the high rate of embryo mortality and the number of infertile eggs laid during this time, we were able to gather trustworthy information on three different types of eggs: (1) infertile eggs, (2) viable eggs, and (3) eggs in which the embryos perished at different stages of incubation.

On Day 1, goose eggs were initially set in the incubator for a period of 2 hours. After that, they were removed and a shell surface T of each egg was measured at an upper middle point for four times: immediately after removing the eggs from the incubator, and then three times with 0.5-hour interval each. An ambient temperature was 26 °C. Such a procedure was repeatedly undertaken over 10 incubation days. At Day 10, the eggs were candled [18,22], and the judgement concerning their fertility and embryo mortality was made (see [18]). After removing infertile eggs and eggs with still (i.e., dead) embryos, the remaining eggs continued to be incubated, while daily measurements were taken of both shell T during 1.5-hr natural cooling and egg weight (W). In addition, the air cell diameter (d) was measured with an accuracy of 0.1 mm, followed up by its conversion into volume ( $V_{ac}$ ) in cm³, according to the method presented in Narushin et al. [24].

Eggs that were found to be infertile on Day 10 were broken, carefully washing the shells of the contents. After air drying to a constant mass, the shell was weighed ( $W_s$ ) with an accuracy of 0.01 g and its thickness (t) was measured using a digital caliper with an accuracy of 0.01 mm at three points: at the equator and closer to the blunt and pointed ends. The averaged result was taken as an actual t of goose eggs.

## 2.3. Mathematical and statistical analyzes

Utilizing Microsoft Excel's computational capabilities and the STATISTICA 5.5 software (StatSoft, Inc./TIBCO, Palo Alto, CA, USA), the data were processed and further analyzed.

#### 3. Results and discussion

After candling on Day 10, it was determined that 52 of the 80 goose eggs involved in the experiment were infertile. Seven eggs with still (i.e., dead) embryos were also detected and, accordingly, the remaining 21 eggs were classified as fertile. All infertile eggs were assigned a quantitative index of 1, the fertile eggs, respectively, that of 2, and the eggs with dead embryos were designated as 3. Thus, the numerical series included 52 ones, 21 twos and 7 threes. The size and weight characteristics of eggs, both before putting into the incubator and during 10 incubation days, are described in detail elsewhere [15].

## 3.1. Shell temperature as a criterion for putting eggs into categories 1 to 3

Since adequate distribution of goose eggs into the corresponding categories of infertile (1), fertile (2) and, conditionally called, "mortal" (3) eggs was carried out on Day 10, the average results of shell *T* measurements for each of the three groups are presented as an example on Days 1, 5 and 10 in Fig. 1. Such selective visualization of the *T* measurement process, according to the beginning, middle and end of the 10-day incubation, enabled to analyze possible changes without oversaturation with graphical dependencies of daily fixations of this parameter.

Analysis of the cooling process of eggs on different incubation days demonstrated almost complete functional coincidence in all three groups. Only by Day 10, there was a certain tendency towards separation of trend lines. Hereby, at the same heating intensity in the incubator, the shell surface of fertile eggs had the *T* values slightly higher than those of infertile and mortal eggs. Moreover, this trend continued during the eggs' natural cooling for 1.5 hours, although these differences were insignificant.

It was notable that after 1.5 hours of natural cooling on Day 1, the shell *T* among eggs of all groups dropped from approximately 36 °C to a little more than 30 °C. Herewith, measurements during later periods showed a much more pronounced decrease in *T* to approximately 28 °C. Obviously, the possible explanation of this phenomenon lies in the structural changes in the egg contents (yolk–white) during incubation, which may affect thermal conductivity.

We hypothesized that differences in egg cooling characteristics may be more pronounced when W is taken into account. In this regard, we decided to use a relative indicator of shell T per 1 g of W, i.e., T/W. The respective results are presented in Fig. 2.

When analyzing the trend lines in Fig. 2, one can notice that the relative *T* of eggs in which the embryos will die over the next 10 days was lower than the average values for the other two groups. Moreover, such differences are most pronounced on Day 1, gradually leveling out during later stages. Although the statistical calculation using the Student's *t*-test did not confirm the significance of these differences between the groups, they were clearly distinguishable visually (Fig. 2a). One can even assume that eggs, for one reason or another, with low thermal conductivity, tended to increase the mortality of their embryos. To ascertain this assumption, we used the Pearson correlation coefficient. Herein, in order not to distort the correlation analysis results by the presence of three groups, we decided to combine Groups 1 and 2 into one, assigning it the number '2'. Indeed, at this stage, our goal was to possibly isolate the mortal group (3) from the entire sample of goose eggs even before they were placed in the incubator.

The highest correlation coefficient value was found when measuring T after 1 hour of natural cooling (-0.104), although it was rather small and insignificant. Graphic visualization of the correlation analysis results (Fig. 3) suggests the clearly insufficient accuracy of this prediction, according to which it was possible to confidently identify only one egg, whose minimum T/W ratio indicated the unsuitability of this egg for subsequent incubation.

Our further attempts to improve the prediction accuracy of the possible distribution of goose eggs into groups by introducing into the dependent indicator such additional values as S, V and D did not result in any improvements in comparison with the T/W values. Note that the D value is an indirect characteristic of the structural components of the egg contents, as evidenced by our previous studies [25] focused on the theoretical and practical aspects of the relationship between egg parameters and D. However, despite the insufficient accuracy of the obtained prediction, the T/W ratio can be used as a separate temperature index in similar studies.

## 3.2. Egg cooling rate

Another T criterion that we decided to explore was called 'egg cooling rate'. The physical meaning of this index is how quickly the shell T drops during the natural egg cooling over a certain period of time. At the same time, we used two cooling rate options: change in T for 0.5 ( $\Delta T_{0.5}$ ) and 1.5 ( $\Delta T_{1.5}$ ) hours. The first indicator ( $\Delta T_{0.5}$ ) was calculated as the difference between successive T measurements divided by 0.5 hours. The second indicator ( $\Delta T_{1.5}$ ) was the difference between the first and last measurements divided by 1.5 hours. Thus, we have a database for three daily parameters of  $\Delta T_{0.5}$  and one daily  $\Delta T_{1.5}$  for 10 days. The graphical dependencies presented in Figs. 4 and 5 help assess the possibility of using them to classify eggs into Groups 1 to 3.

Mathematical analysis of the obtained results did not allow us to identify significant differences in any of the indicators ( $\Delta T_{0.5}$  and  $\Delta T_{1.5}$ ), which is also evidenced by the visualization of the resulting trends (Figs. 4 and 5). The introduction of specific cooling rate indicators, taking into account the values of W, S and/or V, did not improve the outcome.

#### 3.3. Dynamics of changes in shell temperature indicators

In our previous related studies [15], we proposed a fairly effective approach to search for the relationship between the measured parameters of goose eggs and their belonging to one of three incubation categories (Groups 1 to 3). This approach implied analyzing not only the results of direct measurements, but also the nature of their changes over a certain period of time. For these purposes, we used the trend line slope of such indicators, which we designated as TAN, since the slope angle was expressed through a tangent taken in radians.

If we compare the degree of the trend line slopes in Fig. 2 with that in Fig. 4, it was even visually noticeable that it was precisely the nature of the cooling rate change,  $\Delta T_{0.5}$  (Fig. 4), that had more pronounced differences across 10 incubation days, and therefore was more promising for subsequent mathematical processing. However, we analyzed both options and the corresponding visualization is presented in Fig. 6. Both indicators, TANT and TAN $\Delta T_{0.5}$ , were characterized by the distribution of eggs into groups at the end of the 10-day incubation period. However, while the differences for TANT were not significant throughout the entire period, significant differences were already noted for the TAN $\Delta T_{0.5}$  from Day 8. In this regard, the TAN $\Delta T_{0.5}$  indicator was considered more promising for deriving the calculated dependence of the eggs' distribution among the groups. The highest correlation coefficient values were R = 0.505 (p < 0.05) for TAN $\Delta T_{0.5}$  in Groups 1 (infertile) vs 2 (fertile), and R = -0.446 (p < 0.05) in Groups 2 (fertile) vs 3 (mortal), both being noted on Day 9.

For possible practical purposes to sort eggs according to TAN $\Delta T_{0.5}$ , we considered it appropriate to combine eggs unsuitable for incubation (infertile and mortal) into one group under the index '1'. The correlation for this combined group and the group of fertile eggs was 0.491 (p < 0.05), and a visualization of the distribution of these groups within a range of TAN $\Delta T_{0.5}$  values on Day 9 is presented in Fig. 7.

As part of our previous studies [15], we demonstrated the derivation of the optimal approximation formula for data consisting of two categories, which in our case were infertile and mortal eggs (denoted by 1) and fertile eggs (denoted by 2). These were represented by the function y that had the shape of a sigmoid. Then, the approximation of the obtained data shown in Fig. 7, can be expressed by the following functional dependence:

$$y = 1 + \frac{1}{1 + e^{-4.05(\text{TAN}\Delta T_{0.5} + 4.93)}},$$
(1)

where y is the category into which a particular goose egg falls (1 or 2), and, accordingly, TAN $\Delta T_{0.5}$  is the trend line slope of the cooling rate change process for 0.5 hours on Day 9 (in rad).

Visualization of the approximation results is shown in Fig. 8.

However, the calculation using Eqn1 demonstrated insufficiently accurate results, according to which four eggs suitable for incubation in Group 2 (fertile eggs) were mistakenly classified as unfit (Group 1), and 19 eggs were in Group 1 (infertile and mortal) identified as suitable. While the error for Group 1 was only an additional 19 eggs that could have been discarded (and due to inaccurate calculations would be incubated

in vain), four eggs that could have given viable offspring (but were mistakenly removed) already represented serious economic losses. Indeed, this constituted 19% of all fertile eggs in our experiment. In this regard, the accuracy of the calculation had to be increased, if possible.

We attempted to improve the forecast by using several data series. In addition to the data from Day 9, the highest correlation coefficient value was also found for  $TAN\Delta T_{0.5}$  on Day 8; this was equal to 0.367 (p < 0.05). Approximation of the data from Days 8 and 9 allowed us to obtain the following calculation formula:

$$y = 4.704 + 0.27 \text{TAN} \Delta T_{0.5}^8 + 0.893 \text{TAN} \Delta T_{0.5}^9 + 0.095 \text{TAN} \Delta T_{0.5}^8 \cdot \text{TAN} \Delta T_{0.5}^9 - 0.028 \left( \text{TAN} \Delta T_{0.5}^8 \right)^2 + 0.026 \left( \text{TAN} \Delta T_{0.5}^9 \right)^2$$
, (2)

where  $TAN\Delta T_{0.5}^8$  and  $TAN\Delta T_{0.5}^9$  are the trend line slopes of the cooling rate change process over 0.5 hours on Days 8 and 9, respectively (in rad).

The correlation coefficient between the actual values of the numerical series of infertile and mortal (1) and fertile (2) eggs and the values obtained as a result of the calculation using Eqn2 was 0.572 (p < 0.05). Despite the increased prediction reliability degree, improvements affected only Group 1. As a result of using Eqn2, there were three eggs incorrectly identified as suitable. Compared to the 19 eggs incorrectly obtained using Eqn1, this was quite a big improvement. However, the number of misidentified eggs in the more economically important Group 2 went up to 10, or almost 48% under our experimental conditions. Attempts to improve the outcome by using the results of three incubation days (8, 9 and 10) also failed, as did the introduction of additional egg parameters, such as W, S and/or V.

In our previous studies [15], we showed a number of more promising parameters of goose eggs, e.g., such as the dynamics of changes in their D and/or the ratio of  $V_{ac}$  to W, which aided in identifying much more accurately than T fluctuations and at an earlier incubation stage what hatching eggs had defects. As it turned out, T differences began to appear more clearly and significantly by Day 10. There, it seemed worthy of shifting the time factor in searching for the relationship between hatching qualities and the characteristics of the natural cooling process of goose eggs to a later incubation stage.

## 3.4. Mortal eggs after Day 10 and specific temperature index

The generally accepted practice of ovoscoping goose eggs on Day 10 [22,26,27] does not mean that it positively warrants hatchability of the remaining number of eggs. In this regard, the technology of repeated ovoscoping on Day 26 or 27 is often used [22,26,28]. Considering the above described trends in the differences between viable eggs and incubation defects after approximately Day 8, we evaluated the suitability of shell cooling parameters after Day 10, i.e., after all infertile and eggs with dead embryos were

removed from the incubator. As a result, 21 eggs were left, for which we continued the incubation process performing daily candling and T change measurements during 1.5-hour cooling. One dead embryo was found in one of these eggs on Day 14 and six more at once on Day 15. On Day 17, two more embryos died. The last accidental embryo death was recorded on Day 18, after which all the remaining eggs completed the incubation process by producing eleven healthy goslings. Thus, we had the following ratio for the subsequent analysis: 10 eggs entered the mortal group, which we continued to identify under the number 1, and 11 eggs remained in the fertile group, which we decided to call further as 'alive' and assigned it the number 2. We were more interested in finding relationships, if any, between the nuances of the T regime of natural egg cooling during the period of 11 to 13 incubation days, as precursors of a certain round of embryonic mortality, obviously associated with the stage of physiological transformation of the embryo in terms of its transfer to, and preparation for, the hatching stage (e.g., [29]). According to Lasoń and Łukaszewicz [30], the peak of embryo mortality in the middle of incubation is most likely associated with improper closing allantois and increased embryo metabolism.

Similar to what we showed in section 3.1 (Fig. 1), i.e., no differences in *T* during natural cooling among eggs of different categories during the first incubation days, *T* dynamics during Days 11 to 13 was also absolutely identical for Groups 1 (mortal) and 2 (alive) as presented in Fig. 9.

Previously (Fig. 2), we already encountered such an examination situation and were convinced that improvements in assessing the desired parameters would be expectable when switching to specific indicators, e.g., T per 1 g of W (i.e., T/W). Therefore, we decided to use a similar approach for a later incubation period. The most promising results were obtained using the egg T based on W per S, i.e., W/S. Referred to by us as the *specific temperature index* (STI), this indicator will further be implemented in the form of TS/W. The use of the S value is quite logical, since the level of convection and, consequently, the rate of the cooling process depends on the surface area. Visualization of changes in the TS/W indicator during 1.5 hours of natural cooling on Days 11 to 13 days is given in Fig. 10.

The differences in this indicator between Groups 1 and 2 were significant (p < 0.05) for each time interval of T measurements. The correlation coefficients between the numerical series consisting of ones and twos (conforming to mortal and alive eggs) and the TS/W value were in the range of 0.53...0.57 (p < 0.05). Hereby, a slightly higher value of the correlation coefficient was noted at the last T measurement point, i.e., at the end of the 1.5-hour interval of natural egg cooling. However, despite the fairly high correlation between these indicators (see the respective graphical visualization presented in Fig. 11), we were unable to obtain an adequate calculation dependence. This was due to the fact that while eggs of Group 1 (containing mortal embryos) more conformed to higher TS/W values, eggs of the second category (containing alive embryos) were evenly distributed along the entire horizontal scale. Thus, by correctly

identifying eggs of category 1, we may attribute some eggs with similar *TS/W* values to misidentified eggs subject to subsequent erroneous culling.

Therefore, we decided to use cumulative TS/W data for all three chosen incubation days (11 to 13). As a result, the following mathematical relationship was obtained:

$$y = 67243.4 \left(\frac{T_{1.5}^{11}S}{W_{11}}\right)^{3.3} \cdot \left(\frac{T_{1.5}^{12}S}{W_{12}}\right)^{-0.7} \cdot \left(\frac{T_{1.5}^{13}S}{W_{13}}\right)^{-5.8},$$
(2)

where y is the category into which a particular goose egg falls (1 or 2);  $T_{1.5}^{11}$  ...  $T_{1.5}^{13}$  are shell temperature at 1.5 hours after removing eggs from the incubator on Days 11 to 13, respectively (in °C);  $W_{11}$  ...  $W_{13}$  are egg weights on Days 11 to 13, respectively (in g); and S is the surface area of the eggs (in cm<sup>2</sup>).

The correlation coefficient between the obtained data, which we rounded to whole numbers (one or two, respectively) and the actual values was 0.610 (p < 0.05). Using Eqn2, two eggs in each category (1 and 2) were misidentified. Such an error was acceptable for category 1, since it only implied the irrational incubation of two eggs that could not be removed from the incubator in time. On the other hand, an error in Group 2 would entail the culling of potentially alive goslings. Within the total number of eggs in Group 2, this error would be 18%, which is not so small.

To improve the prediction result, we supplemented the initial STI dataset with eggs incubated for few more days. As such, the most promising were Days 8, 9 and 10, and the magnitude of the respective correlation coefficients for TS/W values were smaller (0.29 ... 0.52) than those on Days 11 to 13, although they were significant (p < 0.05). Moreover, we were able to somewhat improve the prediction and increase the correlation by taking, as initial values, the average values of four T shell indicators, i.e., immediately after removing the eggs from the incubator, and at the next three measurement points (every 0.5 hour of their natural cooling). As a result, the following computation equation was inferred:

$$y = 3050 \left(\frac{T_{av}^8 S}{W_8}\right)^{7.3} \cdot \left(\frac{T_{av}^9 S}{W_9}\right)^{20.8} \cdot \left(\frac{T_{av}^{10} S}{W_{10}}\right)^{-25.8} \cdot \left(\frac{T_{av}^{11} S}{W_{11}}\right)^{-4} \cdot \left(\frac{T_{av}^{12} S}{W_{12}}\right)^{6.3} \cdot \left(\frac{T_{av}^{13} S}{W_{13}}\right)^{-6.6}, \tag{3}$$

which, through simple mathematical transformations, can be easily represented in a simpler form:

$$y = \frac{3050}{S^2} \cdot \left(\frac{T_{av}^8}{W_8}\right)^{7.3} \cdot \left(\frac{T_{av}^9}{W_9}\right)^{20.8} \cdot \left(\frac{T_{av}^{10}}{W_{10}}\right)^{-25.8} \cdot \left(\frac{T_{av}^{11}}{W_{11}}\right)^{-4} \cdot \left(\frac{T_{av}^{12}}{W_{12}}\right)^{6.3} \cdot \left(\frac{T_{av}^{13}}{W_{13}}\right)^{-6.6}, \tag{4}$$

where (in Eqns 3 and 4) y is the category into which a particular goose egg (1 or 2) falls;  $T_{av}^{8}$  ...  $T_{av}^{13}$  is average shell temperature measured at intervals of 0.5 hours for 1.5 hours after removing eggs from the incubator on Days 8 to 13, respectively (in °C);  $W_{8}$  ...  $W_{13}$  are egg weights on Days 8 to 13, respectively (in g); S is the surface area of the eggs (in cm<sup>2</sup>).

The correlation coefficient between the data calculated using Eqn2, which we rounded to whole numbers (one or two, respectively) and the actual values was 0.772 (p < 0.05). As a result, two eggs in the mortal embryo category (1) were misidentified. However, eggs in the more important category (2), suitable for further egg incubation, were identified absolutely correctly. That is, from the sample remaining after checking on Day 10 and consisting of 21 goose eggs, 8 can be discarded before the embryos in them begin to die. The remaining two eggs remained among the good ones. However, the timely and correct prediction of 80% of eggs whose embryos are not viable is a fairly high result.

Additionally, we made further attempt to improve the prediction result by combining T indicators with a number of other parameters that were successfully implemented in our previous experiments [15]. These included the dynamics of changes in D and the ratio of  $V_{ac}$  to W (i.e.,  $V_{ac}/W$ ). However, we were unable to get any improvements. Probably, the differences in the values of parameters D and  $V_{ac}/W$  between Groups 1 and 2 were more pronounced in the first incubation stages, enabling to predict early embryonic mortality. Later mortality cases can be predicted more accurately by using egg T scores, being significant after Day 8. Moreover, W (used in defining STI, i.e., TS/W) was included in both additional parameters D and  $V_{ac}/W$ , which, obviously, did not lead to an improvement in the calculation result. A natural question arises: what is all this mathematical prediction for, if it is possible to check the status of the embryo using an ovoscope?

When studying the reliability of ovoscoping goose eggs, Lasoń and Łukaszewicz [30] and Łukaszewicz et al. [27] showed that observational error can be quite significant. The only verification alternative would be to open the egg. However, this procedure does not allow for further incubation of eggs. Thus, the use of such an additional alternative indicator as T change in the course of natural cooling when eggs are briefly removed from the incubator may be an indispensable approach. Like an "arbitrator", it will allow to make the right decision on whether further incubation of this particular egg makes sense. In addition, it is very important to make the right decision at least a few days before the embryo dies.

In this regard, another natural question would be whether daily, albeit short-term, regular cooling of eggs can harm the process of embryogenesis. Judging by the practice of natural incubation when eggs are hatched by wild species, daily cooling only benefits the embryos. According to Cooper and Voss [8], "parents regulate brooding conditions by balancing the thermal requirements of embryos with time spent

away from the nest for self maintenance." This peculiarity was used by many researchers when assessing the effectiveness of temporary egg cooling in various poultry species during the incubation process, confirming the feasibility of such a technological solution [31–36]. For goose eggs, cooling them during incubation is especially important [22]. Thus, monitoring and recording the process of *T* changes in shell cooling can significantly facilitate the process of identifying goose eggs with dead embryos in the middle of egg incubation.

What was the cause of such differences in the STI values (TS/W) between the categories of eggs with mortal (1) and alive (2) embryos? If you look at the dynamics of changes in the average values of this indicator over 17 days of observation (Fig. 12), the differences between Groups 1 and 2 were noticeable from Day 1. From Day 2 to Day 17, these differences were significant (p < 0.05).

Obviously, the differences were due to differences in the metabolic level of developing embryos. According to Meijerhof and van Beek [6], T in the incubator does not always correspond to T of the embryo. Lourens et al. [37] suggested that embryo development and hatchability are more influenced by embryo T than by air T. Based on data from Nangsuay et al. [38], it can be assumed that the release of excess heat by the embryo is the result of ineffective conversion of energy and protein into body tissue. That is, the more heat the embryo produces, the less efficiently its body uses its nutrients. Thus, such inefficiency results can be seen in embryonic mortality at later stages of incubation.

However, while conventional measurements of shell surface T did not reveal discrepancies in their values (Fig. 9), the use of STI (TS/W) was an important aid in obtaining significant results in predicting the viability of goose embryos.

# 3.5. Prediction of egg shell parameters

It is quite logical to assume that the natural egg cooling process is somewhat influenced by the shell indicators. The thicker and/or denser it is, the longer it obviously retains heat, preventing cooling. In themselves, such shell parameters as t and  $W_s$  are extremely relevant for assessing the egg quality, especially if they can be determined using non-destructive methods [39]. Therefore, we decided to evaluate how T indicators, along with morphological parameters of goose eggs, can be used for accurate and non-destructive quality control of their shells.

Since the shell is a calcium source for the developing embryo, its thinning has been observed during incubation in various bird species (e.g., [40–42]). In this respect, the analysis of shell characteristics in our study was only possible in the Group of infertile eggs. It can be assumed that the eggshells of this particular Group remained unchanged. However, the shells of eggs in which the embryos were dead could be subject

to thinning during the days while the embryos were alive. Thus, to analyze a possible prediction of shell parameters, we utilized a sample of 28 goose eggs.

Since the values of t and  $W_s$  were of interest to us before the eggs were placed in the incubator, we could only use those predictive parameters that, accordingly, could be measured immediately after laying. The respective parameter set, along with the correlation coefficients with the shell parameters, is listed in Table 1. Moreover, considering the data as calculated above, we took into account of our correlation analysis – not only the data of the initial measurements, but also their various combinations – as well as the dynamics of changes during the measurement process.

Analysis of the data in Table 1 enabled to conclude that the  $W_3$  was a more predictable parameter than t. Herewith, the W<sub>s</sub> values closely correlated with both the physical and geometric parameters of eggs and their T indicators. As for t, its values tended to be more closely related to the egg parameters than to the T measurement values and their corresponding mathematical transformations. As we expected, relationships between different measurements allowed us to strengthen the correlation coefficients, although a rather interesting opposite effect was observed in some cases. For example, D (calculated as W/V) showed a more than 1.5-fold increase in the correlation coefficient with the t value (0.448) in comparison with the correlation of the initial parameters W and V (-0.252 and -0.327, respectively). At the same time, the relationship between D and  $W_s$  (0.396) turned out to be approximately two times lower than when compared with W or V (0.843 and 0.792, respectively). We also achieved the classical synergy effect, i.e., an increased correlation when using a combination of parameters, in addition to D and t. This occurred with the pair of parameters  $T_{av}/W$  and t: the  $T_{av}/W$  ratio showed a slightly higher correlation coefficient with t(0.276) than that for  $T_{av}$  (0.095) and W (-0.252) alone. In contrast to t, a synergistic effect was not obtained for W<sub>s</sub>. Most likely, this was due to the fact that the relationship between the parameters considered was rather logical in nature. Obviously, it makes sense to examine combinations of initial measurements based on the principles of mathematical approximation aimed at achieving the result of maximum reliability between the measured and calculated parameters.

In our previous studies [43–46], we demonstrated the feasibility of improving the accuracy of the prediction of shell parameters (t and  $W_s$ ) when using combinations consisting of products of power functions of the original egg measurements that had the greatest correlations dependencies. We also undertook a similar approach, as a result of which the following mathematical relationships were obtained:

$$W_s = 0.00018W^{2.124} \cdot V^{-4} \cdot S^{3.838} \cdot T_{av}^{0.519} \cdot \Delta T_{1.5}^{-0.058},$$
 (5) with  $R = 0.908$  ( $p < 0.05$ );

$$t = 0.0047W^{1.286} \cdot V^{-2.821} \cdot S^{2.019} \cdot T_{av}^{0.697} \cdot \Delta T_{1.5}^{-0.079},$$
with  $R = 0.593$  ( $p < 0.05$ );

where (in Eqns 5 and 6)  $W_s$  is shell weight (in g), t is shell thickness (in cm), W is egg weight (in g), V is egg volume (in cm<sup>3</sup>), S is shell surface area (in cm<sup>2</sup>),  $T_{av}$  is average shell temperature in 2 hours after eggs' heating in the incubator measured immediately after their removal and in the next 1.5 hours with a frequency of every 0.5 hour (in °C), and  $\Delta T_{1.5}$  is egg cooling rate for 1.5 hours of natural cooling of eggs in 2 hours after their heating (°C/hour).

Visualization of a comparative analysis of the approximate values of shell weight ( $W_{s\ ap}$ ) and thickness ( $t_{ap}$ ) vs. actual values ( $W_{s}$  and t, respectively) is provided in Fig. 13.

For both shell parameters ( $W_s$  and t), the most accurate prediction was achieved with a "symbiosis" of physical, geometric and T indicators of goose eggs. To assess how much T data facilitated the calculation accuracy, we approximated the measurements of  $W_s$  and t using only combinations of parameters W, V and S. As a result, the following dependencies were obtained:

$$W_s = 0.0008W^{2.226} \cdot V^{-4.075} \cdot S^{3.863},$$
 (7) with  $R = 0.904$  ( $p < 0.05$ );

$$t = 0.0354W^{1.452} \cdot V^{-2.934} \cdot S^{2.026},$$
 with  $R = 0.563$  ( $p < 0.05$ ). (8)

Comparison of Eqns 5 and 6 with their truncated versions, Eqns 7 and 8, respectively, suggested, on the one hand, a low degree of contribution of shell T parameters to the prediction accuracy of its quantitative characteristics ( $W_s$  and t). On the other hand, even a small improvement is sometimes quite relevant, especially for analytical studies. Both pairs of equations (Eqns 5 and 6, and Eqns 7 and 8) work quite well and, therefore, the choice of a particular one can be left to the discretion of the user, the tasks set and the expected results.

#### 4. Conclusions

In the present investigation, we proposed a non-destructive technique for predicting whether an embryo is still (i.e., dead) or alive within an incubated goose egg. The conducted research allowed us to establish promising goose egg indicators that were obtained as a result of measuring the T of their shells immediately after removal from the incubator, and then every 0.5 hour during the natural cooling process.

The most effective T indicator called STI was a combination of three parameters: shell T divided by the ratio of W to S (i.e., TS/W). We found that all four T measurements made on a daily basis during the natural egg cooling can be used in calculating STI. However, we would recommend using  $T_{av}$  of all measurements taken as the most integral and significant T indicator. Using STI (TS/W) calculated on Days 8 to 13, we were able to correctly reject 80% of goose eggs in which the embryos died, starting from Day 14 until the completion of the hatching process.

The combination of parameters included in the specific temperature index (T, S and W) also demonstrated its practicality in deriving dependencies for calculating  $W_s$  and t of goose eggshells. The only thing in this case is that we recommend additionally using another T indicator, "egg cooling rate". The physical meaning of this criterion is how quickly the shell T drops during the first 1.5 hours of the natural egg cooling of the egg ( $\Delta T_{1.5}$ ). The integrated use of all four parameters made it possible to achieve high calculation accuracy as expressed by correlation coefficients of 0.908 for  $W_s$  and 0.593 for t.

A natural question may arise about the possibility of using the obtained mathematical algorithms for other species of poultry. Most likely, an automatic transfer of these models is not possible. The main reason for that is the difference in geometric dimensions of goose eggs in comparison with other eggs. Larger goose embryos, which have a different level of metabolism than, say, chicken embryos, probably make some characteristic contribution to the temperature characteristics of the egg in the course of the incubation process. Nevertheless, the proposed principles and methodological aspects of the research will certainly prove useful when performing similar work with eggs of other bird species. Thus, the results obtained can form the basis of a computational algorithm for the appropriate actuator, which can step forward as a pioneering engineering solution in poultry farming for egg incubation improvement.

#### **Declaration of competing interest**

The authors declare no conflict of interest

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#### **CRediT** authorship contribution statement

Valeriy G. Narushin: Conceptualization, Data curation, Formal analysis, Methodology, Resources, Software, Visualization, Investigation, Writing – original draft, Writing – review & editing. Michael N. Romanov: Project administration, Validation, Writing – review & editing. Louis Gressier: Investigation. Elouann Jacob: Investigation. Attila Salamon: Investigation, Project administration, Validation, Writing – original draft,

Writing – review & editing. **Sabine Klein:** Investigation, Validation, Writing – review & editing. **John P. Kent:** Investigation, Supervision, Validation, Writing – review & editing.

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#### **Figure captions**

- **Fig. 1.** The natural cooling process of goose eggs for 1.5 hours. Average shell temperatures (*T*) by groups: infertile (1), fertile (2) and mortal (3) on Days 1 (a), 5 (b) and 10 (c) of incubation.
- **Fig. 2.** The process of changing the relative shell temperature per 1 g of egg weight (T/W) during natural cooling of goose eggs for 1.5 hours. Average T/W indicators by groups: infertile (1), fertile (2) and mortal (3) on Days 1 (a), 5 (b) and 10 (c) of incubation.
- **Fig. 3.** Graphic visualization of the distribution of eggs into groups '2' (infertile + fertile eggs, n = 73) and '3' (mortal eggs, n = 7) depending on the T/W value. (a) Y- and X-axes: T/W; (b) Y-axis: egg group, X-axis: T/W.
- **Fig. 4.** The change process of the cooling rate over 0.5 hours ( $\Delta T_{0.5}$ ) during natural cooling of goose eggs. Average indicators by groups: infertile (1), fertile (2) and mortal (3) on Days 1 (a), 5 (b) and 10 (c) of incubation.
- **Fig. 5.** The change process of the cooling rate over 1.5 hours ( $\Delta T_{1.5}$ ) during natural cooling of goose eggs. Average values by groups: infertile (1), fertile (2) and mortal (3) during 10 incubation days.
- **Fig. 6.** Graphical dependences of changes in the average values of TANT (a) and TAN $\Delta T_{0.5}$  (b) among goose egg groups during the first 10 incubation days.
- **Fig. 7.** Graphic visualization of the eggs' distribution into groups '1+3' (infertile + mortal eggs) and '2' (fertile eggs) depending on the TAN $\Delta T_{0.5}$  value.
- **Fig. 8.** Approximation of TAN $\Delta T_{0.5}$  data on Day 9 by function (1) (yellow line).
- **Fig. 9.** The natural cooling process of goose eggs for 1.5 hours. Average shell temperatures (*T*) by groups: mortal (1) and alive (2) on Days 11 (a), 12 (b) and 13 (c).
- **Fig. 10.** The process of changing the specific temperature index (TS/W) during natural cooling of goose eggs for 1.5 hours. Average indicators by groups: mortal (1) and alive (2) on Days 11 (a), 12 (b) and 13 (c).
- **Fig. 11.** Graphical visualization of the specific temperature index (*TS/W*) values for eggs from mortal (1) and alive (2) groups as measured immediately after removal from the incubator on Days 11 (a), 12 (b) and 13 (c).
- Fig. 12. Changes in the specific temperature index (TS/W) over 17 incubation days on average for the groups of mortal (1) and alive (2) eggs.
- **Fig. 13.** Visualization of the comparative analysis: (a) approximated shell weight values ( $W_{s ap}$ ) according to Eqn5 vs actual values ( $W_s$ ); (b) approximated shell thickness ( $t_{ap}$ ) according to Eqn6 vs. actual values (t).