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# THERMOGRAPHIC MEASUREMENTS OF EGGSHELL TEMPERATURE DURING INCUBATION

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**Abstract**

*The aim of this work was to study changes of eggshell temperature (EST) during short-time egg cooling using thermography measurements in order to monitor a course of chick embryo development. Eggs (n = 600, broiler line ROSS 308) were incubated in Petersime setter S576 in temperature (T = 37.8℃) and relative humidity (RH = 51%). The EST was measured with the infrared camera Thermovision A20 (FLIR Company) from the first (E1) until the 19th (E19) days of embryogenesis. The series of three EST measurements was performed outside the incubator (T =22.4℃, RH 75%), i.e. immediately (EST-0), and then at 15 (EST-15) and 30 minutes (EST-30) following the eggs withdrawal from the setter.*

During consecutive days of incubation EST-0 of alive embryos steadily increased. At E1 it was 37.0 ± 0.45°C (mean ± SE) and from E8 it increased significantly above 38°C (i.e. to 38.1 ± 0.47°C; P≤0.05). The highest value of EST-0 (39.6 ±1.02°C) occurred at E16 (P≤0.05). From E1 to E16 the difference between EST-0 and EST-15 was about 3.0°C (P>0.05) while at E19 it was only 1.8 ± 0.43°C (P≤0.05). This observation indicates that during the hatching period the thermoregulation system of chick embryo is already partly developed and active.

All the three ESTs of eggs with the dead embryos were always lower in comparison with ESTs of the alive ones. These differences of ESTs exceeded 0.5°C from E8 (P≤ 0.05), and allowed to detect eggs with mortalized embryos.

In conclusion, results of the experiment showed that thermography is a useful method for non-invasive monitoring of incubation process and the proper development of the embryo.

***Key words:*** THERMOGRAPHY, EGG SHELL TEMPERATURE, CHICK EMBRYO DEVELOPMENT

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## Introduction

It is known that in the incubation process of domestic avian eggs the development of the embryo is mainly influenced by the physical microenvironment surrounding the egg (Van Brecht *et al* 2005). But on the other hand, the temperature experienced by the developing avian embryos depends on the three factors: 1) the air temperature, 2) the exchange of heat between the egg and its microenvironment and 3) the time-variable heat production of the embryo (French 1997).

The air temperature at an incubator is a very important factor for pre- and post- hatching chicken development. The optimal air temperature for the embryo development is defined as 37.5ºC to 37.8ºC, but some authors consider that an internal egg temperature is more important (embryo temperature, ET) (Joseph *et al* 2006, French 1997). The first signals of heat producing by a chick embryo were observed at the 3rd day of incubation but intensive heat production begins on the 10th day of incubation (Lourens *et al* 2006 a,b). However, ET measuring is problematic because the structural integrity of shell is damaged, which causes the risk of contamination and damage to developing embryo (French 1997). This problem can be solved by measuring of the surface temperature of egg shell (EST) which is the border layer between the external and internal environment of avian embryo (French 1997, Lourens *et al* 2005). EST is generally measured pointwise by thermistor (Lourens *et al* 2005) or infrared thermometer (pyrometer) (Dobrzański and Bednarczyk 1988, Joseph *et al.* 2006). Another method of infrared radiation (IR) monitoring is thermovision (thermography). An infrared (thermovision) camera measures and images the emitted infrared radiation from a whole object. The fact that radiation is a function of an object surface temperature makes it possible for the camera to calculate and display this temperature. This method is used in engineering, building and human and veterinary medicine. The application of this method to monitor the changes in egg shells temperature during incubation was tested by Augustyn *et al* (2006). During this experiment it was shown that the EST of developing eggs increased during the incubation but the temperature of the shell was not identical on the whole surface. The shell of living egg above an air chamber was significantly lower than the rest of the surface of the egg. These local differences were more detectable in the case of undeveloped and dead eggs.

The aim of this work was to study the changes of eggshell temperature (EST) during short-time egg cooling using thermography measurements in order to monitor a course of chick embryo development.

## Material and methods

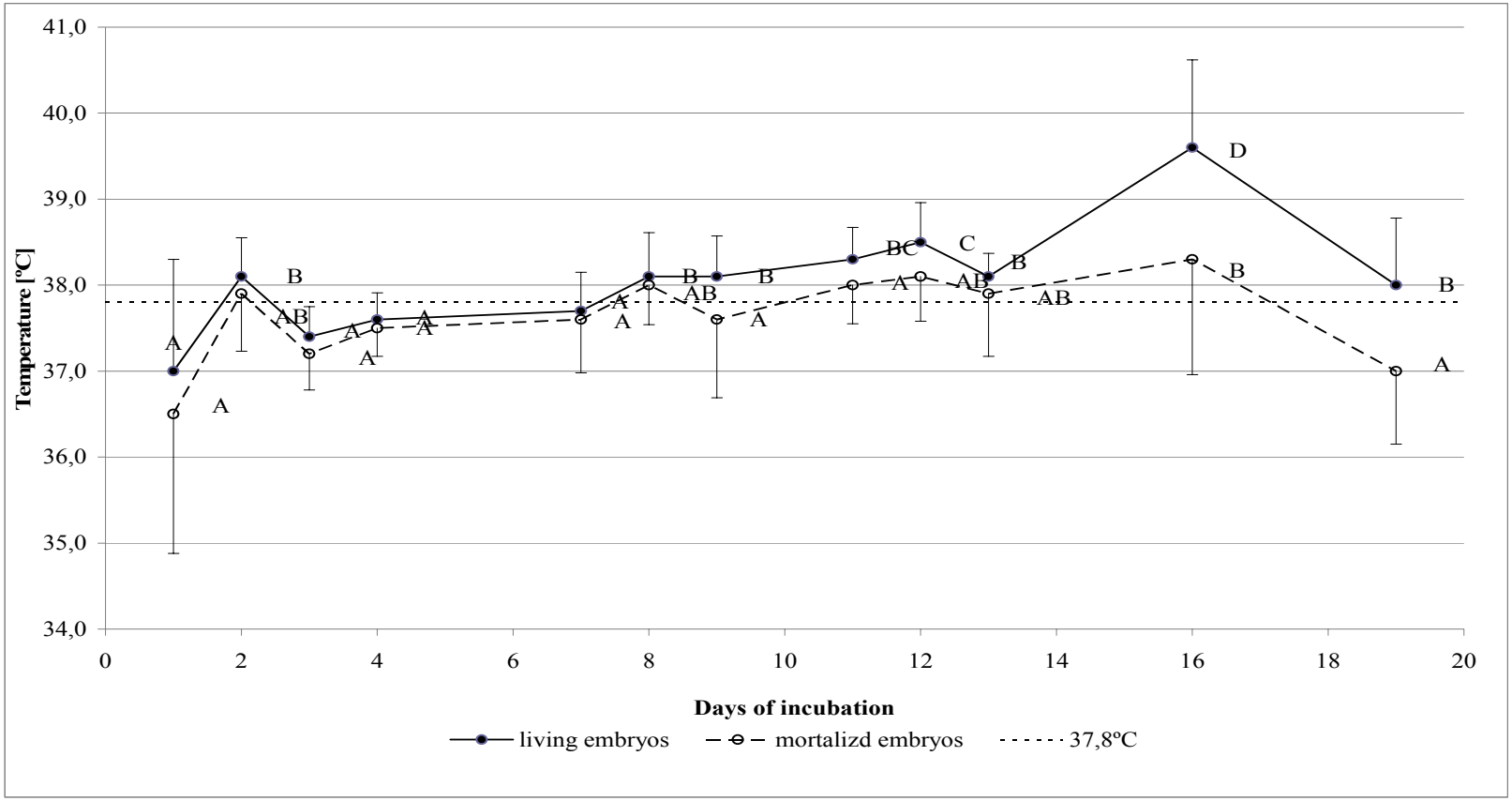
The experiment was conducted at a commercial hatchery. Eggs (n = 600, broiler line ROSS 308, mean weight of egg ± SD: 63.2 ± 5.69g) were individually numbered and incubated in Petersime setter S576 in temperature (T) 37.8°C and relative humidity (RH) 51%. The egg shell temperature (EST) was measured with the infrared camera Thermovision A20 (FLIR Company) on 1st (E1), 2nd (E2), 3rd (E3), 4th (E4), 7th (E7), 8th (E8), 9th (E9), 11th (E11), 12th (E12), 13th (E13), 16th (E16) and 19th (E19) days of incubation. The series of three EST measurements was performed outside the incubator in a setters hall at constant air parameters: T 22.4°C, RH 75%), i.e. immediately (EST-0), and then at 15 (EST-15) and 30 minutes (EST-30) following the eggs withdrawal from the setter. The results of the measurements were presented on the thermographs, where temperature distribution was shown in the color scale. The EST's values of each egg were taken from equatorial region of egg shell.

During the experiment the eggs were candled on E10 but the mortalized eggs were remained on the trays and embryopathological analysies were made after hatching.

The data obtained were analysed statistically by three-way analysis of variance followed by Duncan's multiplerange test. The statistical analysies were performed with the SigmaStat 2.03 computer program (SPSS Inc., USA).

## Results and discussion

The air temperature in a setter (machine temperature, MT) amounted to 37.8 ± 0,1 ºC and it seems that the egg shell temperature (EST) of incubating eggs should be similar. However, the results of measurements show that difference between MT and EST depends on the stage of embryogenesis and oscillates from -1.8ºC to 1.9ºC [Fig 1].




Figur 1

| | 0 | 1 | 2 | 3 | 4 | 7 | 8 | 9 | 11 | 12 | 13 | 16 | 19 | 20 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| living embryos | | 37,0 | 38,1 | 37,4 | 37,6 | 37,7 | 38,1 | 38,1 | 38,3 | 38,5 | 38,1 | 39,6 | 38,0 | |
| mortalizd embryos | | 36,5 | 37,9 | 37,2 | 37,5 | 37,6 | 38,0 | 37,6 | 38,0 | 38,1 | 37,9 | 38,3 | 37,0 | |
| SD | | 1,3 | 0,45 | 0,35 | 0,31 | 0,45 | 0,51 | 0,47 | 0,37 | 0,46 | 0,27 | 1,02 | 0,78 | |
| SD | | 1,62 | 0,67 | 0,42 | 0,33 | 0,62 | 0,46 | 0,91 | 0,45 | 0,52 | 0,73 | 1,34 | 0,85 | |
| 37,8ºC | 37,8 | 37,8 | 37,8 | 37,8 | 37,8 | 37,8 | 37,8 | 37,8 | 37,8 | 37,8 | 37,8 | 37,8 | 37,8 | 37,8 |

Some authors consider that this difference can come up to 4-5ºC (French 1996, Joseph *et al* 2006, Lorens *et al* 2006).

Value of EST-0 (mean ± SD) on E1 was 37.0 ± 1.30ºC and increased by about 0.1 at each day of incubation. The increase of EST-0 during the first 10 days of chick embryo development probably resulted from the progress of chorio-allantois membrane (CAM) and its vascular system (Romanoff 1960). The chorion and alantois originate between $50^{th}$ and $69^{th}$ hour of incubation, on E5 these two membranes fuse and on E8 - E10 cover the whole egg from inside (Romanoff 1960). The carried out thermograph measurements indicated that at this moment EST-0 overstepped a border 37.8ºC (Fig 1).

During the following days of incubation the EST-0 increased to 39.6 ± 1.02ºC on E16, but on E19 significantly lowered to 38.0 ± 0.78ºC (Fig. 1.). Keeping of EST on the level above 37.8ºC is probably caused by intensification of embryo's metabolism (French 1997, Deming and Ferguson 2006). It is possible because the CAM (from E10) takes over the respiration function from a yolk sack (Deming and Ferguson 2006, Lourens *et al.* 2006) and the oxygen utilization rapidly increases (Vleck and Vleck 1987, Vleck and Hoyt, 2004). At this stage of chick embryogenesis the heat production is about 25-30mW, but on E15-16 it attains the level of 137-155 mW (Romijin and Lokhorst 1960, Lorens *et al* 2006a). Lorens *et al* (2006) consider that such heat production lets the EST increase to above 5ºC more than MT. The occurred - lower of EST-0 – the E19 level [38,0ºC (Tab.3, Fig.1)] may be explained by observation of the heat production which at this period decreased to 130 mW (Romijin and Lokhorst 1960, French 1997, Deming and Ferguson 2004). However, Augustyn *et al* (2006) supposed that this phenomenon results from the lost of an albumen and an amniotic fluid, high caloric capacity substances, swallowed by chick embryo during prehatching period (E18-E19).

The incubation technology of avian eggs enjoins to candle eggs and to cull unfertilized and dead ones. During this operation eggs stay outside the setter in T about 22-25ºC and RH 65-70%. The present study occurred that 15 minutes cooling of eggs during first two weeks of incubation resulted in lowering of EST at about 2.7 - 3.6ºC (Fig. 2.) and 30 minutes cooling at about further 1.7 - 2.5ºC . These results show that during this period the heat production was insufficient for keeping internal temperature of egg (Romijin and Lokhorst 1960, Lorens *et al*.2006b). Interesting is that the highest decrease of EST (4,2 ± 0,80ºC after 15 minutes, $P \leq 0.05$ and 2.2 ±0,49ºC after following 15 minutes, $P \leq 0.05$) was observed at the moment of intensification of embryo's metabolism (E16). It seems that at this period an embryo is particularly sensitive to the thermal manipulation and disturbance of incubation parameters. However the decrease of EST on E19 after 15 minutes of cooling was only 1.8 ±0,55ºC ($P \leq 0,05$) but further 2.2 ± 0.78 ($P > 0.05$) after following 15 minutes (Fig. 2. The obtained results show that the faster loosing of heat takes place at the first quarter of cooling what is in agreement with the observation of Van Brecht *et al*. (2005). Moreover it can confirm the thesis that the thermoregulation mechanisms of chick begin work at last stage of embryogenesis (Deming i Ferguson 2004, Lorens *et al*. 2006ab, French 1997).

In this study was observed that the EST of eggs with the dead embryos was subjected to the similar alteration as EST of eggs with the living embryos but their EST was always lower. (Fig 1, 2). Differences between EST-0 during first eight days of incubation oscillated from 0.1°C to 0.2°C ($P > 0.05$), but on E9 this value overstepped 0.5ºC ($P \leq 0.05$) and from E16 was higher than 1ºC ($P \leq 0.05$) (Fig. 1.). Moreover, during cooling, only in the second half of embryogenesis the decrease of EST of dead embryos eggs was higher about 1.5-1.7ºC than living embryos eggs ($P \leq 0.05$) (Fig 2.).

Similar results were found earlier by Augustyn *et al* (2006) - they considered that thermography method could be used to cull the eggs with embryos mortalized in advanced stages of embryogenesis because the embryos which were dead in the second half of incubation were not identified in standard candling.

These decreases were only weakly correlated with the stage of embryogenesis: -0,153 ($P \leq 0.05$) and -0.018 ($P \leq 0.05$), respectively.

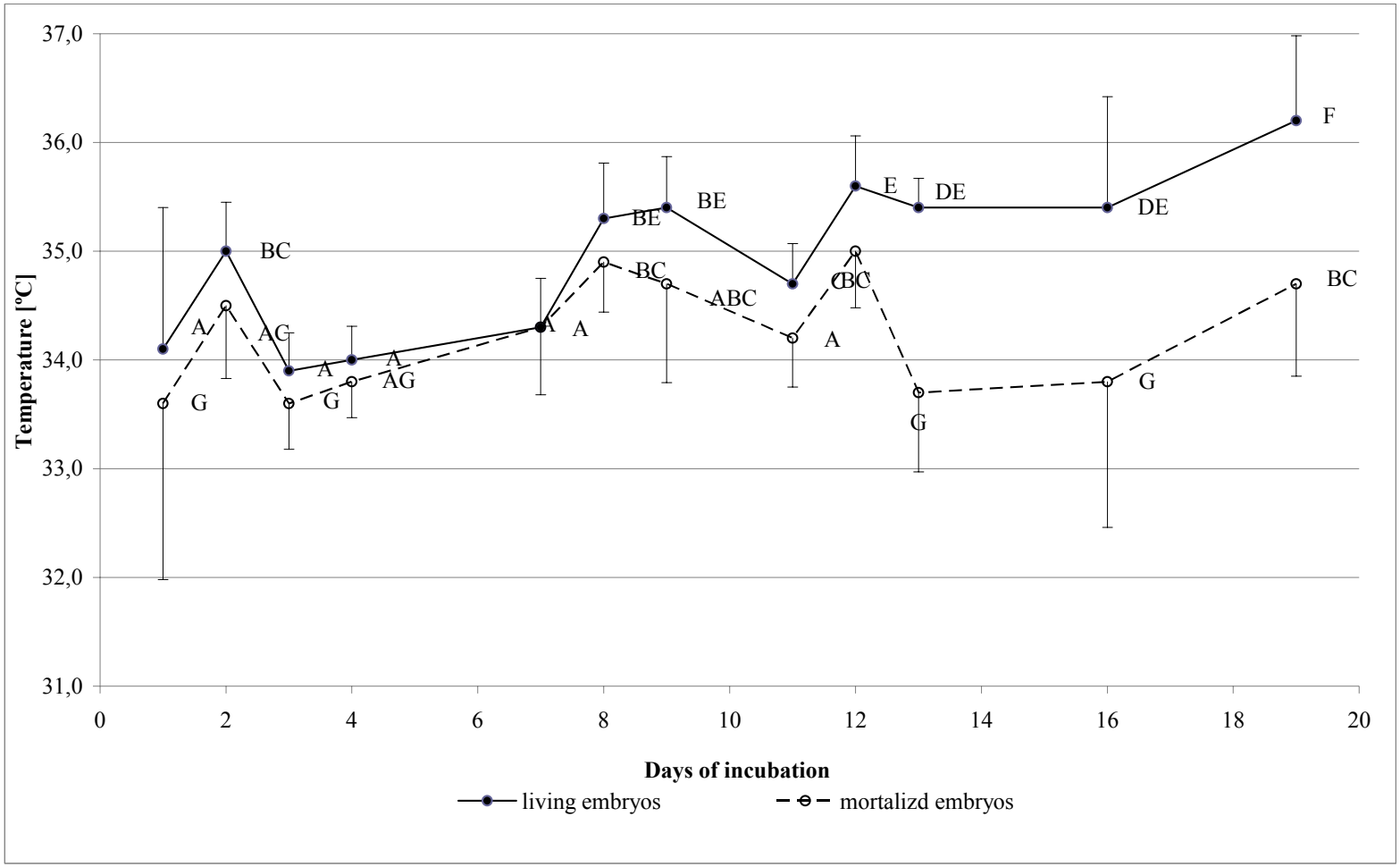



Figure1. Egg shell temperature of no-cooling eggs.

Figur 1

| | 0 | 1 | 2 | 3 | 4 | 7 | 8 | 9 | 11 | 12 | 13 | 16 | 19 | 20 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| living embryos | | 34,1 | 35 | 33,9 | 34 | 34,3 | 35,3 | 35,4 | 34,7 | 35,6 | 35,4 | 35,4 | 36,2 | |
| mortalizd embryos | | 33,6 | 34,5 | 33,6 | 33,8 | 34,3 | 34,9 | 34,7 | 34,2 | 35 | 33,7 | 33,8 | 34,7 | |
| SD | | 1,95 | 0,95 | 0,63 | 0,57 | 1,12 | 0,84 | 0,82 | 0,53 | 0,76 | 0,45 | 1,15 | 1,01 | |
| SD | | 2,06 | 1,35 | 0,9 | 0,75 | 1,18 | 0,83 | 0,95 | 0,66 | 0,82 | 1,73 | 1,3 | 1,18 | |
| 37,8ºC | 37,8 | 37,8 | 37,8 | 37,8 | 37,8 | 37,8 | 37,8 | 37,8 | 37,8 | 37,8 | 37,8 | 37,8 | 37,8 | 37,8 |
| | | $34,1^{A}$ | $35,0^{BC}$ | $33,9^{A}$ | $34,0^{A}$ | $34,3^{AD}$ | $35,3^{BE}$ | $35,4^{BE}$ | $34,7^{CD}$ | $35,6^{E}$ | $35,4^{BE}$ | $35,4^{BE}$ | $36,2^{F}$ | |
| | | $33,6^{ABC}$ | $34,5^{ABC}$ | $33,6^{A}$ | $33,8^{A}$ | $34,3^{ABC}$ | $34,9^{BC}$ | $34,7^{ABC}$ | $34,2^{ABC}$ | $35,0^{BC}$ | $33,7^{ABC}$ | $33,8^{A}$ | $34,7^{ABC}$ | |

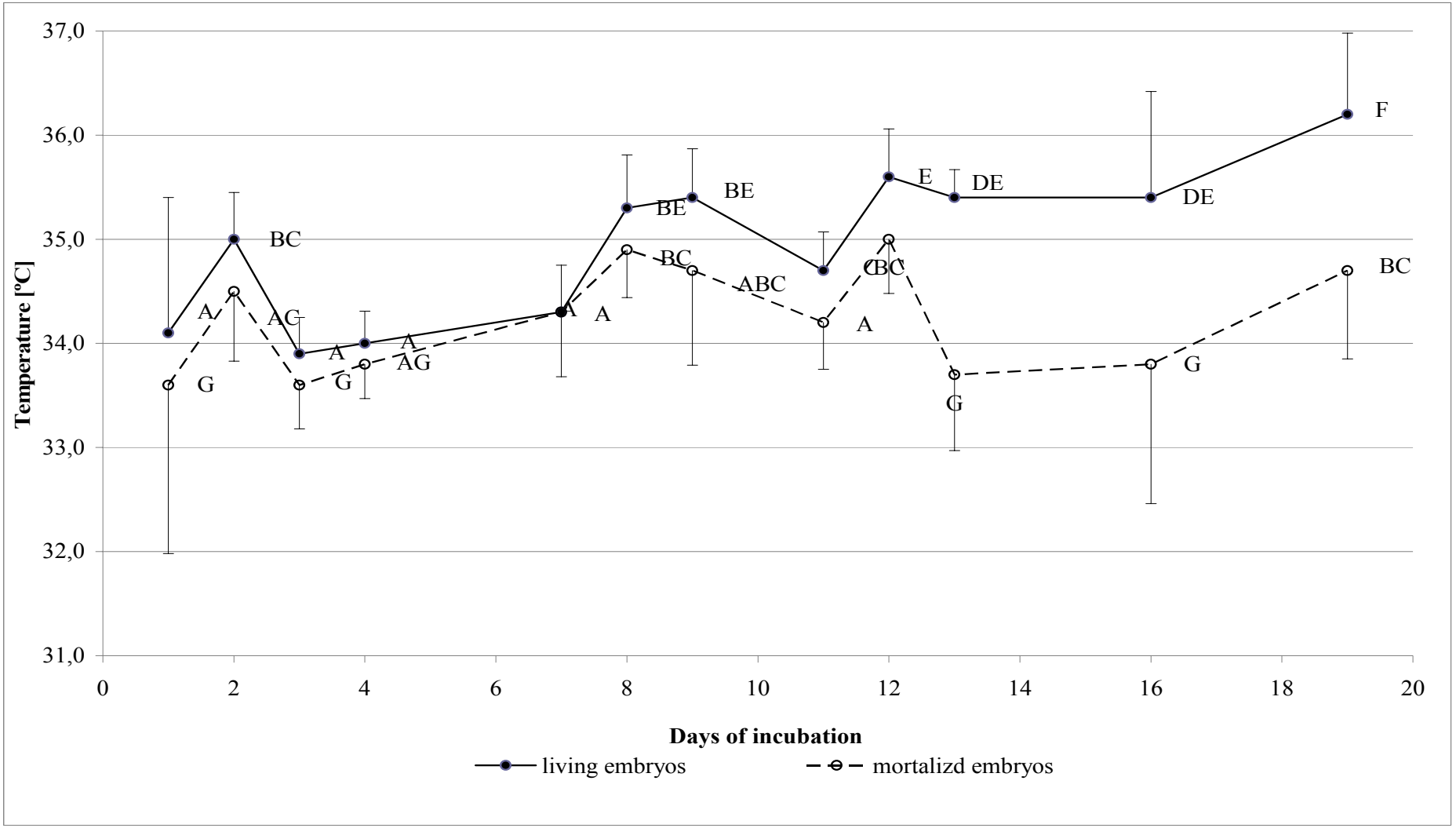



Figure2. Egg shell temperature of 15-minutes cooling eggs.

Figur 1

| | 0 | 1 | 2 | 3 | 4 | 7 | 8 | 9 | 11 | 12 | 13 | 16 | 19 | 20 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| living embryos | | 34,1 | 35 | 33,9 | 34 | 34,3 | 35,3 | 35,4 | 34,7 | 35,6 | 35,4 | 35,4 | 36,2 | |
| mortalizd embryos | | 33,6 | 34,5 | 33,6 | 33,8 | 34,3 | 34,9 | 34,7 | 34,2 | 35 | 33,7 | 33,8 | 34,7 | |
| SD | | 1,95 | 0,95 | 0,63 | 0,57 | 1,12 | 0,84 | 0,82 | 0,53 | 0,76 | 0,45 | 1,15 | 1,01 | |
| SD | | 2,06 | 1,35 | 0,9 | 0,75 | 1,18 | 0,83 | 0,95 | 0,66 | 0,82 | 1,73 | 1,3 | 1,18 | |
| 37,8ºC | 37,8 | 37,8 | 37,8 | 37,8 | 37,8 | 37,8 | 37,8 | 37,8 | 37,8 | 37,8 | 37,8 | 37,8 | 37,8 | 37,8 |
| | | $34,1^{A}$ | $35,0^{BC}$ | $33,9^{A}$ | $34,0^{A}$ | $34,3^{AD}$ | $35,3^{BE}$ | $35,4^{BE}$ | $34,7^{CD}$ | $35,6^{E}$ | $35,4^{BE}$ | $35,4^{BE}$ | $36,2^{F}$ | |
| | | $33,6^{ABC}$ | $34,5^{ABC}$ | $33,6^{A}$ | $33,8^{A}$ | $34,3^{ABC}$ | $34,9^{BC}$ | $34,7^{ABC}$ | $34,2^{ABC}$ | $35,0^{BC}$ | $33,7^{ABC}$ | $33,8^{A}$ | $34,7^{ABC}$ | |

Figur 1

| | 0 | 1 | 2 | 3 | 4 | 7 | 8 | 9 | 11 | 12 | 13 | 16 | 19 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| living embryos | | 33,9 | 32,5 | 31,8 | 31,5 | 32,2 | 33,4 | 33,6 | 33,1 | 33,4 | 33,5 | 33,1 | 34,1 |
| mortalizd embryos | | 33,6 | 32,1 | 31,6 | 31,4 | 32,1 | 32,9 | 33,1 | 32,4 | 33 | 32 | 31,6 | 32,7 |
| SD | | 1,08 | 1,02 | 0,74 | 0,51 | 1,26 | 0,97 | 0,95 | 0,57 | 0,83 | 0,48 | 1,28 | 1,26 |
| SD | | 1,29 | 1,25 | 1,02 | 0,65 | 1,32 | 0,96 | 1,03 | 0,77 | 0,87 | 1,45 | 1,51 | 1,32 |
| średnia | | $33,9^{A}$ | $32,5^{B}$ | $31,8^{BC}$ | $31,5^{C}$ | $32,2^{B}$ | $33,4^{AD}$ | $33,6^{AE}$ | $33,1^{D}$ | $33,4^{D}$ | $33,5^{D}$ | $33,1^{DE}$ | $34,1^{AF}$ |
| SD | | | | | | | | | | | | | |
| średnia | $33,6^{A}$ | $32,1^{ABC}$ | $31,6^{BC}$ | $31,4^{C}$ | $32,1^{ABC}$ | $32,9^{A}$ | $33,1^{A}$ | $32,4^{ABC}$ | $33,0^{A}$ | $32,0^{ABC}$ | $31,6^{BC}$ | $32,7^{AB}$ | |

**Conclusions**

1. The egg shell temperature increases during the following stages of embryogenesis. The increase of it is particularly high after the $10^{th}$ day of incubation.

2. In the beginning stages of incubation the egg shell temperature is lower than the machine air temperature (37.8ºC) and oversteps this board at about the $8^{th}$ day of incubation. In the last days of incubation the surface temperature of egg shell with living embryo amount about 39.6ºC.

3. A short duration (15 minutes) cooling eggs on the $19^{th}$ day of incubation affected significantly the lower decrease of egg shell temperature in comparison with the previous days. It can be supposed that the thermoregulation mechanisms of chick begin to work at the last stage of embryogenesis

4. The thermography method can be used to monitor the chick embryo development and to identify the mortalized embryos in advanced stages of embryogenesis.

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## ТЕРМОГРАФІЧНІ ВИМІРИ ТЕМПЕРАТУРИ ШКАРАЛУПИ ЯЙЦЯ В ПЕРІОД ІНКУБАЦІЇ

Р е з ю м е

1. Температури шкаралупи яйця зростає під час наступних стадій ембріогенезу. Температура зростає найбільше після 10-го дня інкубації.

2. На початкових стадіях інкубації температура шкаралупи яйця нижча ніж температура інкубатора (37,8 ºС) і перевищує цей рівень приблизно на 8 день інкубації. Протягом останніх днів інкубації температура поверхні шкаралупи яйця з живим зародком становила приблизно 39,6 ºС.

3. Короткотривале (15 хвилин) охолодження яєць на 19-тий день мало значний вплив: температура яєчної шкаралупи знизилася порівняно з попередніми днями. Можна припустити, що механізми терморегуляції курчат почниають працювати на останній стадії ембріогенезу.

4. Термографічний метод може бути використаний для моніторингу розвитку ембріонів курчат та ідентифікації загиблих ембріонів на останніх стадіях ембріогенезу.

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## ТЕРМОГРАФИЧЕСКИЕ ИЗМЕРЕНИЯ ТЕМПЕРАТУРЫ СКОРЛУПЫ ЯЙЦА В ПЕРИОД ИНКУБАЦИИ

А н н о т а ц и я

1. Температура скорлупы яйца возрастает во время следующих стадий эмбриогенеза. Больше всего температура возрастает после 10-го дня эмбриогенеза.

2. На начальных стадиях инкубации температура скорлупы яйца ниже чем температура инкубатора (37,8 ºС) и превышает этот уровень приблизительно на 8-й день инкубации. В последние дни инкубации температура поверхности скорлупы яйца с живим зародышем была приблизительно 39,6 ºС.

3. Краткострочное (15 минут) охлаждение яиц на 19 день имело значительное влияние: температура яичной скорлупы понизилась в сравнении с предыдущими днями. Можно предположить, что механизмы терморегуляции цыплят начинают работать на последней стадии эмбриогенеза.

4. Термографический метод может быть использован для мониторинга развития эмбрионов цыплят и идентификации отмерших эмбрионов на последних стадях эмбрионогенеза.

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