# Effects of induced cold stress on eggs during incubation on hatchability, incubation time, and chick quality

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## SUMMARY

The incubation environment, temperature in particular, is a key determining factor influencing embryonic development, hatchability and chick quality in broiler production. This study aimed to evaluate the effects of induced cold stress during the endothermic and exothermic phases of incubation on egg weight loss, incubation duration, hatchability, and day-old chick quality. The objective was to determine whether short and controlled reductions in incubation temperature could improve chick quality without negatively affecting hatching success or incubation time.

In total, 1350 fertile eggs from broiler breeders aged 45 and 46 weeks, representing the peak laying period, were used. Eggs were randomly assigned to three treatment groups. The control group was incubated at standard commercial incubation temperature throughout the incubation period. The first experimental group (E1) was exposed to a cold stress of 25–26 °C for 3 hours starting on day 6 of incubation, from the endothermic phase, until day 18. The second experimental group (E2) was subjected to the same cold treatment beginning on day 14 of incubation, corresponding to the exothermic phase, until day 18. Egg weight loss, incubation time, hatchability, and chick quality at hatch were evaluated. Chick quality was assessed using the Tona scoring system.

Exposure to intermittent cold stress significantly reduced egg weight loss compared with the control group. The average egg weight loss was 10.86% in E1 and 10.99% in E2, while the control group recorded 11.27%. Chick body weight at hatch improved in both experimental groups, with average weights of 62.8 g in the control group, 68.25 g in E1, and 65.9 g in E2. Chick quality scores also improved following cold treatment. The proportion of chicks achieving the maximum Tona quality score was highest in E2 (39.6%), followed by E1 (35.4%) and the control (33.3%). In contrast, hatchability and incubation duration were not affected by the cold exposure, indicating that this controlled cooling procedure did not impair overall incubation performance.

These findings demonstrate that short-term cooling applied at specific phases of incubation, particularly during the exothermic phase, can enhance chick quality and body weight at hatch without affecting hatchability or incubation duration. Given the link between day-old chick quality and later performance, controlled cooling may represent a valuable strategy to optimize incubation practices.

## KEY WORDS

Cold stress, hatcher, egg weight loss, incubation time, hatchability, day-old chick quality.

## INTRODUCTION

The study of factors that influence the production of high-quality chicks is of great interest to hatching egg producers (1). Artificial egg incubation has evolved technologically and economically (2). Remarkable technological and scientific developments have enabled the transition from manual incubation to large-scale incubators and hatchery machines, which incubate a much larger number of eggs with less labor, and increased chick production (3, 4). The principles of artificial incubation were established centuries ago. At this time, the temperature, humidity, and air renewal of the incubation environment, as well as the overturning of the egg were already taken into account. Boleli *et al.* (3) mentioned that eggs were incubated in buildings divided into incubation chambers similar to ovens separated by a central corridor. In the upper part of the egg chamber there were shelves for burning straw, dung, or charcoal to heat the eggs. Vents in the roof allowed the smoke from the fires to escape and provided light. In this primitive incubation system, the temperature in the incubation chambers was managed by controlling the intensity of the fire and opening the manholes, vents, and corridors. Moisture was controlled by placing moisturized jute on the eggs, which were traditionally rotated twice a day. Since then, the means of artificial incubation have evolved. The transition from primitive incubators to sophisticated machines aims to provide appropriate conditions for fertile eggs to produce high-quality day-old chicks (5). Nowadays the supply of day-old chicks is very important for the suc-

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cess of the poultry production chain. Optimizing the quality of this product is the goal of producers. From the fertilized oocyte to placement on the farm, factors such as hatching egg characteristics, incubation conditions, and their interactions can affect chick production and quality. Besides, the management of the incubation parameters is essential for the success of this process (6). This not only highlights the importance of incubation conditions but also offers the possibility of manipulating embryogenesis to improve hatchability and post-hatch performance. Many researchers have tried to improve hatch results and post-hatch performance by manipulating environmental factors during incubation. The most relevant factors influencing the success of the incubation process are temperature, humidity, ventilation, flipping, and the type of incubation equipment used (7, 8). Many studies have focused on examining the effects of increased temperatures during incubation (9, 10). However, few studies have addressed the impact of cold stress during this critical period (11, 12). This research aims to fill this gap by investigating how induced cold thermal stress during endothermic and exothermic egg incubation phases affects egg weight loss rate, incubation time, hatchability, and chick quality to produce higher-quality day-old chicks.

## MATERIALS AND METHODS

### Eggs and experimental design

This study was conducted in the Tunisian Poultry Company, located in Borj Cedria (Tunisia) which is specialized in the production of incubated eggs and one-day-old chicks. The incubation was conducted at two breeds' ages, 45 and 46 weeks, which correspond to the peak laying phase. One thousand three hundred fifty incubating eggs originating from a broiler reproductive center and the same housing unit were used. They were equally divided into three groups: Experimental 1 (E1), Experimental 2 (E2), and Control (C), with three replicates each. All eggs were incubated in a Petersime Vision 576 incubator (Petersime, Belgium) under standardized baseline conditions. They were maintained at a constant setpoint temperature of 37.5 ± 0.2 °C (ranging between 37 and 38 °C), which is considered optimal for broiler embryos to ensure balanced heat transfer and proper embryonic development (13). The relative humidity was regulated at 55-57%, monitored continuously with a calibrated hygrometer, to promote adequate but not excessive water loss through the eggshell. Ventilation was automatically controlled by the incubator system, with fresh air exchange programmed according to the concentration of carbon dioxide ($CO_2$) inside the machine. $CO_2$ levels were allowed to rise gradually during the early incubation period, mimicking natural nest conditions, and were then kept below 0.4% (≈ 4000 ppm) by dynamic adjustment of the air inlets to maintain embryonic metabolism, gas exchange, and acid-base balance (14, 15). This integrated management of temperature, humidity, and ventilation ensured uniform incubation conditions across all groups and replicates. Throughout incubation, these parameters were continuously monitored via the incubator's automated control system. Furthermore, the eggs were incubated at the same time and on the same part of the machine. From day 0 to day 18, eggs were turned hourly at a 45° angle. The experimental groups differed only in the application of a daily controlled cooling period: in group E1, beginning on day 6 of incubation (early endothermic phase), eggs were removed once daily for 3 h at 25-26 °C until day 18, while in group E2, the same cooling protocol was applied daily from day 14 to day 18 (exothermic phase); in contrast, the control group was maintained at standard incubation conditions without thermal manipulation. This mild thermal drop (≈12 °C below setpoint) represents a controlled cold stress rather than refrigeration and is consistent with protocols previously described by Lourens et al. (13), Willemsen et al. (15), and Nyuiadzi et al. (12). During the entire incubation period, the eggs 19$^{th}$ day of incubation, all eggs were transferred to a Petersime Vision P192 hatcher (Petersime, Belgium), where turning ceased and the relative humidity was increased to 65-70% to facilitate hatching.

### Parameters Measured

***Weight loss rate:*** on the first and 18$^{th}$ days of incubation, all hatching eggs were individually weighed to calculate the percentage of weight loss during the incubation period.

***Hatching time and hatchability rate:*** After 512 hours of incubation, the hatcher was opened and the hatching time was recorded at 4 hour-intervals following the first chick hatched. The hatchability rate was determined as the proportion of hatched eggs relative to the total number of incubated eggs.

***Embryonic diagnostic:*** all unhatched eggs were identified by group and their contents were examined macroscopically to categorize them into the following groups: early embryonic mortality, late embryonic mortality, and mortality at pipping. This diagnostic procedure was essential for calculating the loss rates in each treatment.

***Body weight:*** all day-old chicks were collected, numbered, and weighed after the entire batch of chicks had hatched.

***Chick quality:*** At hatch (day 21, after pull), all chicks were individually assessed for quality according to the Tona® scoring system (16). The evaluation included a standardized checklist of parameters: (i) down and general appearance, classified as clean versus dirty and dry versus wet; (ii) navel quality, recorded as completely closed and clean versus partially open, infected, or with unabsorbed tissue; (iii) presence or absence of residual membranes adhering to the body; (iv) presence or absence of residual yolk sac externally visible or palpable; and (v) chick activity, evaluated by responsiveness and vigor (active vs. weak). Each chick was scored immediately after drying in the hatcher, and data collection was performed once at the time of hatch pull, ensuring uniformity across replicates and treatments. The proportion of chicks achieving a perfect score (100/100) and the distribution of sub-scores were then calculated for each group.

### Statistical Analysis

The data were processed with the statistical software package SAS version 9.4 (Statistical Analysis System, Release 9.4 SAS Institute Inc., Cary, NC, USA,2012). As the response variables describing the hatching process were found not normally distributed (Kolmogorov-Smirnov test) and no other distribution was found adequate, the effect of incubation treatment on measured parameters was analyzed using a nonparametric test (Kruskal-Wallis test). In a second analysis, one-day-old quality scores were considered as binomial in the distribution in the chicks of quality scores of 100, on the one hand, and the other, the chicks of quality scores lower than 100. A two-tailed test for comparison of variances was used to analyze the influence of exposure time on the proportion of chicks with a

quality score of 100. A mixed model was used to evaluate the effect of various factors on measured and calculated traits. The following general linear model was used:

$Y_{ij} = \mu + T_i + PH_j + e_{ij}$

Where: $Y_{ij}$ = Calculated traits; μ= the overall average, T = the effect of the Temperature (j= 1-2), PH = the effect of the phase (k= 1-2), and $e_{ijklm}$ = the residual term. The results were given as a least-square means (LSM) with standard errors. A degree of significance of 5% was used. All data are shown as mean-SEM.

## RESULTS AND DISCUSSION

### Weight loss rate

The average weight loss rates recorded were 11.27, 10.86, and 10.99% for the control, E 1, and E 2 groups, respectively (Table 1). The T-test showed that egg weight loss is significantly influenced by heat stress (P=0.02489). The high weight losses in control batches are attributed to the slowdown of the embryonic metabolism due to cooling. Weight loss during incubation is mainly related to water loss. Under incubation conditions, the main energy source of the embryo is the fat contained in the yolk. Their metabolism induces the production of water and $CO_2$, which have been evacuated through the pores of the shell. Egg weight loss during incubation is caused by the evaporation of water through the eggshell pores, which is a crucial process for proper embryonic development (17). However, excessive water loss can negatively affect hatchability and chick quality. Our results are aligned with those reported by Tona *et al.* (14) who achieved the best hatch rates at a weight loss range of 10.9 and 11.1%. However, our findings were of a lower magnitude than those of Salahi *et al.* (5) who reported average loss rates of around 14.20%. Indeed, our results disagree with those of Suarez et al. (18), who reported no significant effect on egg weight loss when eggs were intermittently chilled to approximately 15-16 °C for 6 hours for 6 hours during incubation.

### Hatching rate

The control group had a hatchability rate of 92.6%, while experimental group 1 and experimental group 2 exhibited hatchability rates of 92.4 and 92.8%, respectively. The T-Student test showed no significant difference (P=0.8511) between hatching rates in cold-exposed eggs and those incubated under normal conditions (Table 1). These results suggest that applying cold stress during incubation, whether on day 6 or day 14, did not negatively affect hatchability rates. This may be explained by the timing of the thermal stress application, or by the fact that the intensity and duration of the cooling period were not sufficient to negatively influence hatchability. Indeed, the response of the exposed embryo to low temperatures during the incubation period depends on the age of the embryo, the duration, and the temperature of the exposure (13, 15). While cold stress during incubation is often considered a stressor, mild and controlled cooling events can have a beneficial role by reducing excessive embryonic heat production and balancing gas exchange within the eggshell (13). This process might explain the maintenance of high hatchability rates in the experimental groups.

Our results are similar to those found by Willemsen et *al.* (19) who reported a hatching rate of 92.5% in the Cobb chicken strain, lower than the results of Willemsen *et al.* (15) who reported a hatching rate of 93.1% at an incubation temperature of 37.6 °C, and higher than those reported by Collin *et al.* (20) who found rate of 87.8% for eggs incubated at 37.8 °C. Compared to the control group, the thermal manipulations applied to eggs did not have a significant effect on the hatch kinetics of the chicks (Figure 1). A 25% of the chicks from the control groups were hatched before 24 h and 75% by 20 h. However, 25 and 75% of the chicks under heat stress hatched at approximately 20 and 16 h, respectively. Compared to the control group, the thermal manipulations applied to eggs did not have a significant effect on the hatch kinetics of the chicks.

### Incubation time

The chicks were removed from the hatchery at 512 h of incubation. Our findings indicate that the chicks of the control group and the exothermic group (E2) hatched at 504 hours (8 hours before the end of the incubation) while those of the endothermic group (E1) hatched at 508 hours (4 hours before the end of the incubation) (Figure 1). While these differences in incubation duration were statistically not significant (P=0.0613), it is relevant to consider that thermal manipulation increased the incubation time (Table 1), which is consistent with findings reported in several studies (15, 18, 19). Furthermore, Leandro *et al.* (21) reported that cold stress delays hatching time, with embryos exposed to 32 °C for 5 hours at 16 days of incubation taking approximately 10 hours longer to hatch compared to controls. The delay of hatching from chicks of chilled eggs may be attributed to the slowdown of the embryonic metabolism induced by cooling. The hatching kinetics of the chicks of control and E2 groups were similar, which could be attributed to the fact that during the second stage of embryonic development the exposition of eggs at a temperature of 26 °C for 3 h was insufficient to delay chick hatching. Figure 1 shows that the application of thermal stress during the endothermic phase (E1)

**Table 1** - Impact of induced cold stress on egg weight loss, hatching parameters, chick quality and weight.

| Parameters | E1 | E2 | Control | *P value* |
|---|---|---|---|---|
| Weight loss rate (%) | 10.86 | 10.99±0.08 | 11.27±0.089 | 0.0249 |
| Hatching rate (%) | 92.6±1.3 | 92.4±0.7 | 92.8±0.1 | 0.8511 |
| Incubation time (h) | 510.5±1.5 | 503.5±1.2 | 502±1 | 0.0613 |
| Body weight (g) | 68.2$^{a}$±0.18 | 65.9$^{b}$±0.23 | 62.8$^{c}$±0.15 | 0.0001 |
| Chick quality | | | | |
| • A score of 100% | 35.42$^{a}$ | 39.58$^{b}$ | 33.3 | 0.0458 |
| • Average score | 98.17 | 98.68 | 97.77 | 0.3697 |

E1, experimental 1 group; E2, experimental 2 group. $^{a}$, $^{b}$, different letters indicate statistically significant differences (P<0.05)

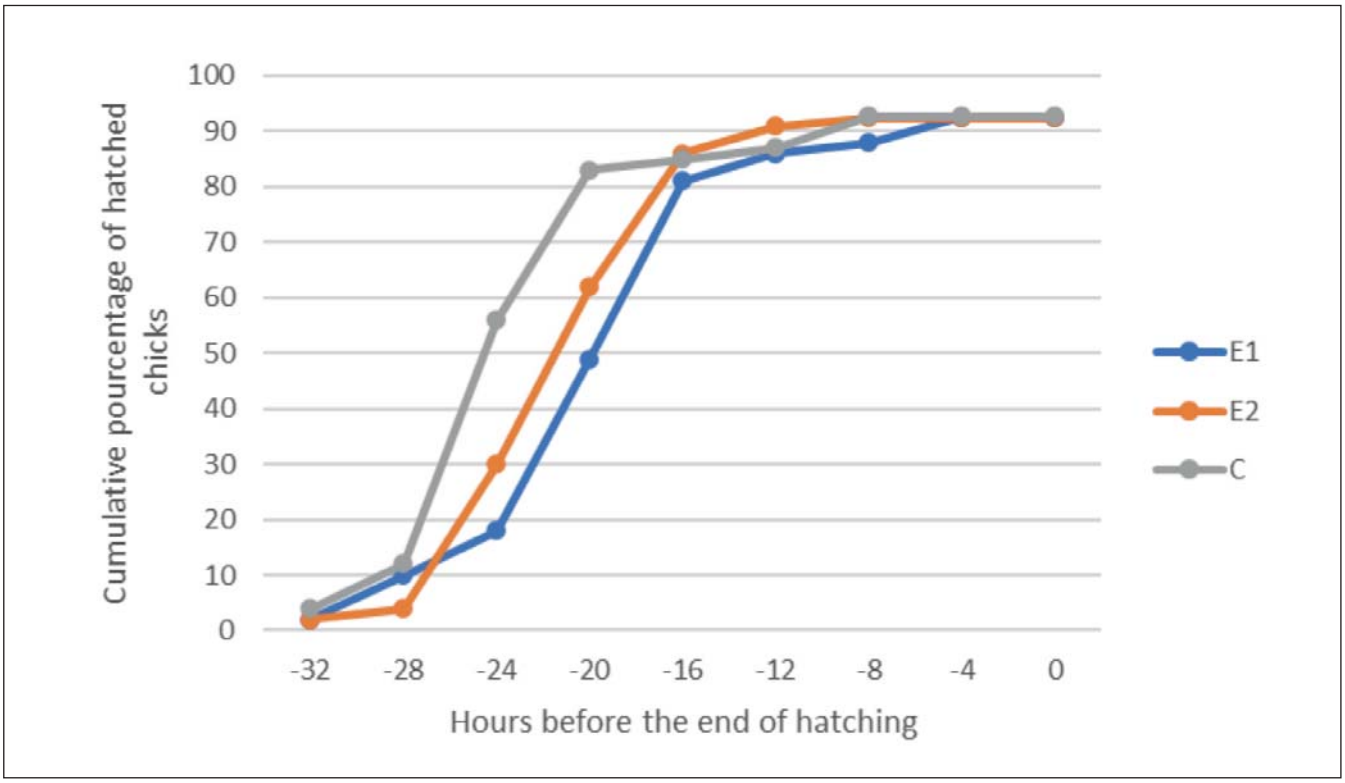


**Figure 1** - Cumulative hatch window profiles of broiler embryos from E1, E2, and C groups under different incubation temperature regimens

had a bigger impact on the chick-hatching duration. Hence, the severity of the effects depends on the duration and intensity of cold exposure. Indeed, Buckland (22) confirmed that temperatures below 11.3 °C cause significant drops in hatchability.

## Chick's body weight

The application of lower temperatures has a high impact ($P < 0.0001$) on the body weight of chicks. The average weight of the chicks is 62.8, 68.2, and 65.9g for the control, experimental 1, and experimental 2 groups, respectively (Table 1). Hence, Chick weight and yield were significantly influenced ($P < 0.0001$) by the type of treatment applied. Chicks exposed to lower temperatures during their embryonic development had a higher weight than the control group. Our results are in agreement with those of Joseph *et al.* (23), who confirmed a significant effect of cold on chick weight. Further, Michels *et al.* (24) reported no difference in the performance of chicks stressed by cold during incubation. Furthermore, a strong negative correlation ($P = 0.0003$) was found between the weight loss rate and the chick weight, which explains the higher weights of the chicks exposed to heat stress. Also, the phase during which the embryo is exposed to lower temperatures can play a major role in the performance parameters. It significantly affects ($P=0.0391$) the weight of the chick. Nevertheless, the highest weights were noted in the batches treated during the endothermic phase ($6^{th}$ day of incubation) (Table 1). These results are in agreement with those of Joseph *et al.* (23) who concluded that lower temperatures during the first 10 days of incubation result in increased chick weight.

## Chick's quality

Chick quality has gained increased importance for hatcheries and also for broiler producers because it has been accepted as an indicator of broiler growth performance. During incubation, the chick quality is affected by some factors. One of the most important factors that affects chick quality is incubation temperature. The present study showed that the Tona score varied among the groups. The percentage of one-day-old chickens with a score of 100 was higher in groups E1 and E2 than in the control group (35.42 and 39.58 *vs.* 33.33, respectively) (Table 1). The highest quality scores of chicks (100) were observed on the chicks of the eggs handled during the exothermic phase ($14^{th}$ day of incubation). Significant differences were observed between the E 2 and the control groups ($P=0.0458$). Regarding average scores, the results indicated that the endothermic group (E2) had the highest average score (98.68), followed by the endothermic group (E1) (98.17) and the control group (C) (97.77) (Table 1). However, these differences were not statistically significant ($P=0.36797$). It is commonly known that the exposure of the embryo to thermal applications plays a major role in the produced chick. However, these results were unexpected, as it was suggested by the literature that small temperature changes would notably affect chick quality. Halle et *al.* (25) reported low-quality chicks incubated at -1 °C for 2 h per day (during the last 6 days of incubation). Moreover, Salahi et *al.* (5) confirmed that exposing eggs during transportation to the hatcher to temperatures below 4 °C increases embryo mortality, malformations, and hatching time. Our results were in agreement with those of Leandro et *al.* (21) who reported that a brief period of cold stress (32 °C for 5 hours) can delay hatching without affecting chick quality. Generally, cold stress increases chick weight at hatching (22). These findings suggest that the impact of cold stress during incubation on chick quality may vary depending on experimental conditions such as the duration and phase of exposure to cold stress.

## CONCLUSION

Chick quality is strongly related to egg characteristics and incubation conditions, which has a direct influence on embryological development and hatchability parameters. This study showed that the application of low temperature reduced egg loss rates, which significantly increased body weight and improved chick quality, by significantly increasing the percentage of day-old chicks achieving a 100% quality score (Tona score). Interestingly, this cold stress did not negatively affect eggs' hatchability rate or incubation time. These findings provide valuable insights into optimizing incubation conditions to enhance chick quality without compromising productivity.

### Acknowledgement
The authors would like to thank Tunisian Poultry Company for their generous support in conducting the experiment in their hatcher.

### Conflict of interest
The authors declare that there is no conflict of interest.

### Author contributions
Mariem Saidani: Data curation, Formal analysis, Methodology, Writing - original draft, Writing - review & editing. Amani Askri: Formal analysis, Methodology, Writing - review & editing. Manel Ben Larbi: Conceptualization, Methodology, Writing - review & editing. Naceur M'Hamdi: Data curation, Software, Supervision, Visualization, Writing - review & editing. Amal Hazmi: Formal analysis, Methodology, Adnene Haffar: Conceptualization, Writing - review & editing.

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