JWPR
Journal of World's Poultry Research
2025, Scienceline Publication
J. World Poult. Res. 15(1): 1-22, 2025
Research Paper
DOI: https://dx.doi.org/10.36380/jwpr.2025.1
PII: S2322455X2500001-15
ISSN: 2322-455X

Effects of Six-Hour Pre-Incubation Thermal Conditioning and Prolonged Storage on Egg Quality, Embryogenesis, Hatchability, and Post-Hatch Physiology of Plymouth Rock Hybrid Chickens in Tropical Climate of Ghana

Sasu Prince[1]*, Agbehadzi Richard Koblah[1], Ackah Edna Mariam[2], Adjei-Mensah Benjamin[1], Felicia Emmanuella Ellison[2], Danquah Cynthia Amaning[3], Tona Kokou[1], Hamidu Jacob Alhassan[2], and Were Pitala[1,4]

[1]*Regional Center of Excellence in Poultry Science (CERSA), University of Lomé, P.O. Box 1515, Lomé, Togo*
[2]*Department of Animal Science, Faculty of Agriculture, College of Agriculture and Natural Resources, Kwame Nkrumah University of Science and Technology, PMB, Kumasi, Ghana*
[3]*Department of Pharmacology, Faculty of Pharmacy and Pharmaceutical Sciences, College of Health Sciences, Kwame Nkrumah University of Science and Technology, PMB, Kumasi, Ghana*
[4]*School of Agronomy, University of Lomé, P.O. Box 1515, Lomé, Togo*

*Corresponding author's E-mail: psasu.research@gmail.com

Received: December 22, 2024, Revised: January 25, 2025, Accepted: February 20, 2025, Published: March 30, 2025

ABSTRACT

Prolonged storage negatively impacts incubation outcomes in commercial hatchery operations, highlighting the need for efficient storage strategies. This study assessed the impact of prolonged storage durations and six-hour pre-incubation thermal conditioning (PTC) on egg quality, embryonic development, hatchability, chick quality, blood profile, and thermoregulation. A total of 2,000 fertile eggs were collected from a flock of 72-week-old Plymouth Rock hybrid hens and subjected to a 2 × 2 factorial design, involving storage for either 14 or 21 days, with or without the application of 6-hour PTC. Following storage, the eggs were incubated in a Jamesway P5000 set at a temperature of 37.5°C and relative humidity of 56%, then transferred on incubation day 18 to a hatcher set at 36.5°C and 60% until hatching. Results revealed that prolonged egg storage without PTC significantly diminished egg protein while PTC effectively countered this decline, enhancing Haugh unit values and blastoderm diameter. Prolonged egg storage without PTC also resulted in increased relative egg weight loss (REWL), fluctuated daily eggshell temperature, and reduced embryonic growth during incubation while PTC significantly reduced these effects, with embryos demonstrating significantly enhanced growth. Additionally, while fertility rates remained stable across all treatments, PTC significantly reduced mortality and improved hatchability by 11.4% in 14-day stored eggs and 10.8% in 21-day stored eggs. It also shortened incubation time, increased post-hatch chick body weights and enhanced their hematological and serum profiles, including normalized thyroid hormone (T3 and T4) levels compared to the non-PTC (control) group. Pearson correlation showed that longer incubation time was positively correlated with higher rectal temperature, serum glucose, and thyroid hormones, but negatively correlated with hemoglobin, mean corpuscular hemoglobin, and total protein in non-PTC chicks. In conclusion, six-hour pre-incubation thermal conditioning mitigates the negative effects of prolonged egg storage and enhances embryogenesis, hatchability, chick quality, blood profile, and thermoregulation in Plymouth Rock hybrid chickens.

Keywords: Embryonic development, Extended egg storage, Plymouth rock hybrid chicken, Pre-incubation thermal conditioning, Thermoregulation

INTRODUCTION

Poultry production depends heavily on optimizing factors that influence embryogenesis and chick quality (Tona et al., 2003). Refining egg handling processes is essential to meet the growing demand for high-quality chicks, as they directly impact egg quality, hatchability, and post-hatch outcomes (Gharib, 2013). Proper storage is a critical component of egg handling, facilitating efficient egg collection and transportation coordination to ensure a consistent supply of day-old chicks (Underwood et al., 2021). Prolonged storage poses significant challenges,

To cite this paper: Sasu P, Agbehadzi RK, Ackah EM, Adjei-Mensah B, Felicia EE, Danquah CA, Tona K, Hamidu JA, and Were P (2025). Effects of Six-Hour Pre-Incubation Thermal Conditioning and Prolonged Storage on Egg Quality, Embryogenesis, Hatchability, and Post-Hatch Physiology of Plymouth Rock Hybrid Chickens in Tropical Climate of Ghana. *J. World Poult. Res.*, 15(1): 1-22. DOI: https://dx.doi.org/10.36380/jwpr.2025.1

especially in tropical climates where high temperatures accelerate the natural deterioration of eggs (Adriaensen et al., 2022). This extended storage negatively affects embryonic viability, disrupts gas exchange (Reijrink et al., 2010), and leads to nutrient loss due to alterations in the eggshell (Özlü et al., 2018). The negative impacts of prolonged egg storage are intensified by the genetic variability among chicken breeds, which differ in their resilience to adverse conditions (Küçükyılmaz et al., 2012). This genetic diversity influences eggshell characteristics, nutritional content, and developmental potential, leading to breed-specific responses to prolonged storage (Scott and Silversides, 2001). Moreover, the interplay between genetic factors and environmental conditions, particularly in tropical climates, complicates the situation further (Zita et al., 2009). High temperatures typical of tropical environments can compromise eggshell integrity, disrupt gas exchange, and accelerate the breakdown of egg components, including albumen, reducing its viscosity and ability to support embryonic development (Fernandes et al., 2023). Elevated temperatures promote lipid oxidation in the yolk, reducing the nutritional quality and shelf life of stored eggs (Suresh et al., 2015). Consequently, chicken eggs stored under such conditions may lose up to 3.67% of their weight within just ten days (Jin et al., 2010; Wang et al., 2017) and experience a significant decline in hatchability (Chen et al., 2005). These issues are particularly concerning for breeds such as the Plymouth Rock hybrid chicken, known for its adaptability and genetic resilience (Kong et al., 2016), which is commonly raised in tropical climates, especially in Ghana (Guo et al., 2019). Despite their resilience, the heat and humidity fluctuations inherent to these regions adversely impact their physiological processes, accelerating egg degradation during storage (Varguez-Montero et al., 2012; Adegbenro, 2023) and further reducing chick viability (Yamak et al., 2020). Therefore, it is crucial to explore strategies to mitigate the negative effects of storage, particularly for these breeds that are commonly raised as commercial layers.

Given the unique challenges posed by elevated temperatures and prolonged egg storage in tropical climates, it is crucial to explore strategies that mitigate these negative effects, especially for breeds like the Plymouth Rock hybrid chicken, which is commonly raised as a commercial layer. One promising strategy to mitigate the negative effects of prolonged egg storage is the application of short incubation periods during storage, referred to as pre-incubation thermal conditioning (PTC) in the present study. This technique mimics the natural incubation behavior of brooding hens (Damaziak et al., 2018). By briefly exposing stored eggs to controlled warmth, PTC reactivates key metabolic processes, helping to counteract the detrimental effects of extended storage (Nicholson et al., 2013). Previous studies have demonstrated that PTC can enhance hatchability and chick quality by stimulating physiological processes that prepare eggs for incubation (Al-Samrai and Al-Dhanki, 2017; Areaaer and Ibrahim, 2019). However, most research on PTC has focused on broiler chickens under temperate conditions, with shorter exposure durations, typically around four hours, applied over shorter storage periods. This creates a gap in understanding its effectiveness in layer breeds like Plymouth Rock hybrids, which are commonly raised in tropical climates of Ghana. In this region, extended storage periods combined with higher temperatures and humidity further challenge egg quality, potentially altering the effectiveness of PTC compared to temperate conditions.

To address this gap, the current study extended the application of PTC from the typical four hours to six hours, with storage periods prolonged up to 21 days. The objective was to assess its impact on egg quality, embryonic development, hatchability, chick quality, blood profile, and post-hatch thermoregulation in Plymouth Rock hybrid chickens. This is particularly relevant as no previous studies have explored the efficacy of longer PTC durations in this breed under tropical conditions. The six-hour PTC was chosen to provide a more sustained thermal activation that counteracts the accelerated egg deterioration seen in tropical climates, where higher temperatures and longer storage periods reduce hatchability.

MATERIALS AND METHODS

Study area and ethical considerations

The experiment was conducted at the Olympio Hatchery in the Department of Animal Science, Kwame Nkrumah University of Science and Technology (KNUST) in Kumasi, Ghana, a facility equipped for avian research. All experimental procedures adhered to the ethical guidelines approved by the Animal Research Ethics Committee (AREC) of KNUST, ensuring that animal welfare was prioritized throughout the study (Quality Assurance and Planning Unit, POLICY 0016, 2018). This included monitoring the handling of eggs and ensuring that all protocols were designed to minimize stress and discomfort to the experimental chicks involved.

Experimental design and treatment allocation

A 2 × 2 factorial design was applied in a completely randomized framework. The factors included two PTC levels (6-hour PTC versus non-PTC) and two storage durations (14 versus 21 days), resulting in four experimental groups: 6hr-PTC × 14d, non-PTC × 14d, 6hr-PTC × 21d, and non-PTC × 21d. Two additional control groups (3 and 7 days of storage without PTC) served as industry baselines, based on prior research (Ansah et al., 2023) in Black-tailed hybrid chickens.

Egg collection and cold storage conditions

A total of 2,000 fertile eggs were collected from 72-week-old Plymouth Rock hybrid hens at Baffour Farms, Kumasi, Ghana. Eggs were randomly selected within a weight range of ± 0.5 g to maintain uniformity and collected early in the morning to reduce handling time. To align with the designated storage durations, 800 eggs were collected first for the 21-day group, followed by another 800 eggs for the 14-day group one week later. Additionally, 200 eggs were collected each for the 7-day and 3-day groups. For the two longer storage periods, the eggs were equally divided into two groups, one for pre-incubation thermal conditioning and the other as a control group. All eggs were stored under controlled conditions at 16°C and 75% relative humidity.

Pre-incubation thermal conditioning procedure

The pre-incubation thermal conditioning (PTC) process involved several key steps, as illustrated in the schematic flow chart in Figure 1. Eggs were first retrieved from storage conditions (16°C and 75% humidity) and pre-warmed in the incubation corridor at ambient temperatures (26°C-28°C) for 1 hour. They were then placed in a setter incubator at 37.5°C and 56% humidity for 6-hour thermal conditioning. After the thermal conditioning, the eggs were pre-cooled in the incubation corridor for 1 hour before being returned to the cold storage room. Eggs designated for the 14-day storage group received a single PTC treatment on day 10, while the 21-day storage group received two PTC sessions on days 10 and 14. Control eggs remained in a cold storage room without undergoing any thermal conditioning.

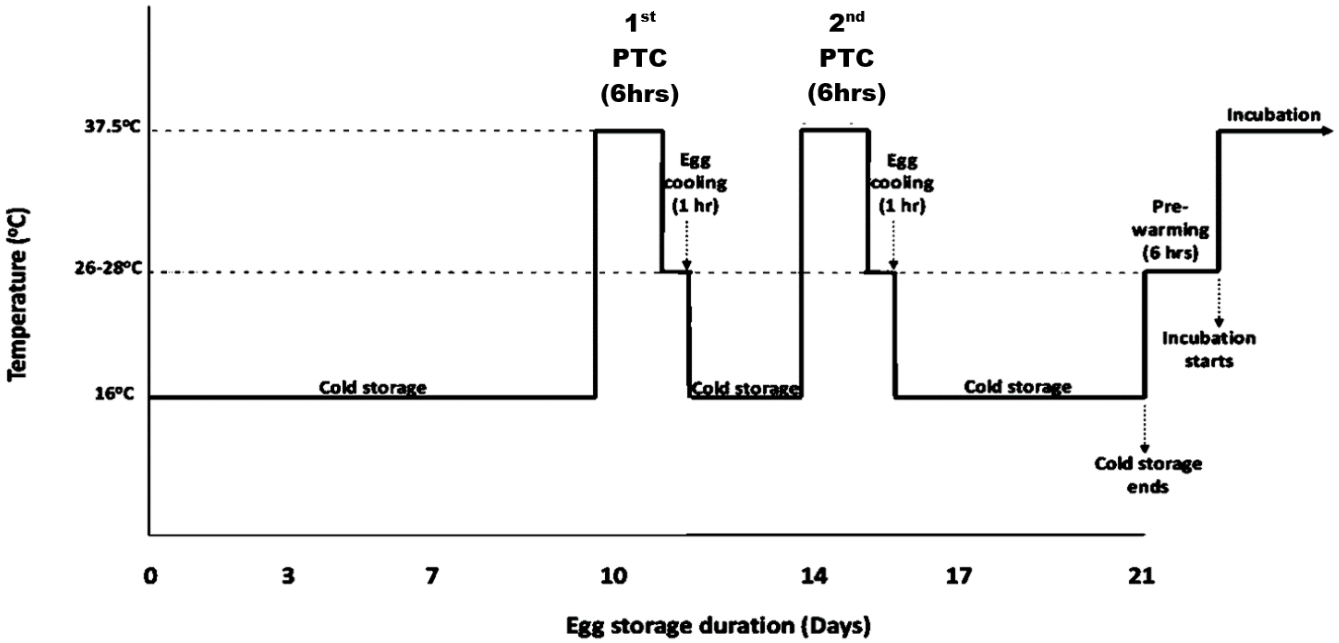

Figure 1. Schematic flow chart of pre-incubation thermal conditioning procedure. Adopted and modified from the earlier procedure. Source: Ansah et al. (2023).

Basic egg quality assessment after egg storage

At the end of the storage and PTC periods, a comprehensive quality assessment of the eggs was conducted before incubation. Each egg was weighed initially and again after the respective storage durations using a precision electronic balance (Model: Ohaus Navigator, Ohaus Corp., USA). The difference between these two weights was expressed as a percentage and recorded as relative egg weight loss during storage. To further assess egg quality parameters, five eggs were randomly selected from each treatment group and carefully cracked open onto a flat surface. The contents were separated into yolk, albumen, and shell, and each component was weighed individually. Eggshell thickness

was measured using a digital micrometer screw gauge (Model: Mitutoyo 293-240, Japan), while the height of the thick albumen surrounding the yolk was measured with the depth gauge of a digital Vernier caliper (Model: Mitutoyo 500-196-30, Japan). The fresh eggshells were weighed, recorded as wet eggshell weight, then washed under running water to remove albumin residues, air-dried for 24 hours, and reweighed to obtain the dry eggshell weight. The fresh yolks were weighed and noted as wet yolk weight, placed in pre-weighed aluminum foil, and dried in a laboratory oven (Model: Memmert UN55, Memmert GmbH + Co. KG, Germany) set at 60°C for four days to facilitate moisture evaporation. After drying, they were reweighed and recorded as "dry yolk weight." The Haugh unit (HU), an established measure of egg protein quality (Haugh, 1937), was calculated using the albumen height and initial egg weight, as described in the Formula 1.

$$Haugh\ unit = 100 \times log(h - 1.7\,w^{0.37} + 7.6) \quad (1)$$

Where h represents the albumen height and w is the egg weight.

Incubation process

Following the respective storage durations and the application of PTC, only the eggs stored for 14 and 21 days were incubated. Before incubation, the eggs were prewarmed for six hours in the incubation corridor, where ambient temperatures ranged from 26°C to 28°C. After prewarming, the eggs were divided into two groups of 1,000 eggs each and placed onto egg trays. These trays were then randomly assigned to two separate setter incubators (Model: P5000, Jamesway Chick Master Ltd, USA), which served as replicated experimental facilities. The incubation process was carried out under controlled conditions of 37.5°C and 56% relative humidity. The incubators were equipped with automatic turning mechanisms that rotated the eggs every hour to ensure even temperature distribution across all surfaces.

Egg weight loss, embryo growth, and metabolic heat production assessments during incubation

The egg weight loss was calculated by weighing two randomly selected egg trays both at the start of incubation (W_0) and on incubation day 18 (W_{18}). The difference in the two weights was used to calculate relative egg weight loss (REWL), expressed as a percentage of the initial weight, using the following formula 2. The external eggshell temperatures were monitored from incubation days 1 to 18 (ID1-18) using a digital infrared thermometer (Model: YI-400, Wenzhou Yosun Medical Technology Co., Ltd, China). The thermometer was calibrated for accuracy and aimed at the external eggshells within the incubator, allowing non-contact temperature measurements that reflected the internal metabolic heat production of the developing embryos (Agyekum et al., 2022). To ensure accuracy and consistency, two representative egg trays from each treatment group were randomly selected from the two separate setter incubators and tagged for all subsequent temperature measurements. Additionally, the temperature within the setter incubators was monitored and recorded daily. The embryo growth assessment was specifically assessed on IDs 4, 7, 11, 14, and 18 as described by Willemsen et al. (2011). Five eggs from each treatment group were randomly selected and cracked open. The embryos were carefully separated from the yolk, weighed, and recorded as “wet embryo weight”. Embryo lengths were measured by extending the embryo from the tip of the beak to the tip of the middle toes using a divider, and the lengths were transferred to a laboratory ruler (Model: 300 mm Stainless Steel Ruler, Mitutoyo Ltd, Japan). After measurements were taken, embryos were oven-dried at 60°C following the procedure described above to remove moisture and subsequently weighed to determine their dry weights.

$$REWL = \frac{(W_0 - W_{18})}{W_0} \times 100 \quad (2)$$

Hatching process and performance assessments

After the embryo assessments, the hourly turning of the eggs continued until day 18, at which point it was discontinued to allow for candling and transfer into the hatcher incubators, preparing the embryos for hatching. To evaluate hatching performance, the fertility rate, embryonic mortality, and hatchability were assessed. The first candling was conducted on day 10 of incubation to differentiate fertile eggs from those experiencing early embryonic death. This process enabled the reliable identification of infertile eggs by visualizing the development of embryonic membranes or color changes in the yolk due to embryonic activity. Infertile eggs were opened to confirm the absence of development. A second candling was performed on day 18 of incubation to assess embryo viability; eggs showing no signs of live embryos were opened to confirm embryo death. Viable eggs were then transferred into hatching baskets and placed in two separate hatcher incubators (Jamesway P5000, USA), set at 36.5°C and 60% relative humidity, to complete the hatching process. On day 22, all hatched chicks were removed from the hatcher, and unhatched eggs were opened to confirm embryo death. The incubation duration,

fertility rate, hatchability, and embryo mortality rates for each treatment group were calculated using the following formulas.

$$Fertility\ rate\ (\%) = \frac{Number\ of\ fertile\ eggs\ on\ day\ 10}{Total\ number\ of\ eggs\ set} \times 100 \quad (3)$$

$$Incubation\ Duration\ (hours) = Day\ of\ Hatch - Day\ of\ Set \quad (4)$$

$$Embro\ mortality\ (\%) = \frac{Number\ of\ dead\ embryos}{Total\ number\ of\ fertile\ eggs} \times 100 \quad (5)$$

$$Hatchability\ (\%) = \frac{Number\ of\ chicks\ hatched}{Total\ number\ of\ fertile\ eggs} \times 100 \quad (6)$$

Post-hatch chick quality assessments

Immediately after hatching, the chicks were gathered and transferred to a designated holding room, where they were organized into their respective treatment groups for a series of quality assessments focusing on critical aspects of body and skeletal development, as well as yolk absorption and navel quality. These assessments were repeated on the seventh-day post-hatch. On both assessment days, five chicks from each treatment group were randomly selected, and their body weights, lengths, and shank lengths were measured. Navel quality was evaluated using the PASCAR scoring system, as described by Rocha et al. (2013) and Yeboah et al. (2019). This system assesses factors such as the presence of navel strings, buttons, and the overall healing process. Chicks with fully closed and clean navels received a score of 1, while those with discolored navels, openings larger than 2 mm, leaking, or attached navel strings were assigned a score of 2. The number of chicks in each category was tallied and expressed as a percentage of the total number of hatched chicks.

Post-hatch physiological assessments

Before and during the quality assessments, the chicks underwent a series of physiological evaluations, which included measurements of rectal temperature, hematological analyses, serum biochemistry, and thyroid hormone assessments, as described below.

Rectal temperature assessments

The rectal temperature of the chicks was measured immediately upon their arrival in the room and subsequently recorded hourly for the first 24 hours. Five chicks from each treatment group were randomly selected for this assessment, with their rectal temperatures recorded using a digital cloacal thermometer (Model: Omron Flex Temp Smart, Omron Healthcare, Japan). Each chick was gently restrained in an upright position with minimal force, ensuring both wings and legs were secured to prevent movement, following the method described by Agyekum et al. (2022). This assessment was conducted to evaluate their thermoregulation capacity and ability to adapt to ambient temperature variations within the holding room.

Blood sampling and laboratory assessments

Blood samples were taken for hematological and serum biochemical analyses by adhering to protocols established by Maxine (1961) and Alonge (2017). Hematological analysis provided insights into oxygen transport, immune function, and overall health. Serum biochemistry evaluated organ function, protein metabolism, and energy utilization, with optimal levels signifying effective nutrient processing crucial for post-hatch development. For both hematology and serum analyses, five chicks from each treatment group were randomly selected. Approximately 5 mL of blood was drawn from 5 chicks in each treatment group. The samples were split, with half placed in EDTA tubes for hematological analysis and the other half in plain red-top tubes for serum biochemistry analysis. All samples were appropriately labeled and transported in a cooler with ice packs to the Main Research Laboratory of Ghana Veterinary Services Directorate, Kumasi-Amakom Division, in line with the transport guidelines outlined by Barde (2022). In the lab, total red blood cell (RBC) was analyzed using a hemocytometer, as reported by Campbell (1995). The packed cell volume (PCV) was determined using the microhematocrit technique following the procedure of Oguntoye (2018). Haemoglobin (Hb) concentrations were analyzed using the cyanmethemoglobin method, as detailed by Simaraks (2004). Mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC), were calculated based on the RBC, PCV, and Hb values, as described by Ritchie et al. (1994). For serum analysis, blood samples were centrifuged at 3,000 rpm for 15 minutes and the resultant serum was further analyzed. Serum total protein and albumin were quantified as described by Varley et al. (1980) and Doumas et al. (1971) respectively. Globulin was calculated by subtracting albumin from total protein as suggested by Barde (2022). Serum total cholesterol was evaluated using the enzymatic end-point method according to Roschlau et al. (1974), and triglycerides were measured using the colorimetric technique described by Bowers and Wong (1980). Glucose concentrations were assessed using the Glucose Oxidase/Peroxidase-Aminoantipyrine-Phenol (GOD/PAP) reagent method as reported by Trinder (1969). Finally, two key thyroid hormones, Tri-iodothyronine (T3) and Thyroxine (T4) concentrations were assessed using the Vitek Immuno Diagnostic Assay System (VIDAS), applying the enzyme-linked fluorescent assay (ELFA) technique described by Favresse et al. (2018). All samples were analyzed within a single assay batch to ensure consistency.

Statistical analysis

For the basic egg quality assessment data collected after storage, a one-way ANOVA was performed while data collected during incubation and post-hatch were analyzed using a two-way ANOVA. All data were analyzed using the generalized linear model (GLM) procedure in SAS version 9.4. Treatment means were compared using the Student Newman-Keuls (SNK) test, with significance determined at $p < 0.05$. The statistical model used to analyze the data was $Y_{ijk} = \mu + \alpha_i + \beta_j + (\alpha \times \beta)_{ij} + \varepsilon_{ijkY}$. Where *Yijk* represents the measured response (parameters) for either egg or chick. The term μ refers to the overall mean of all observations, providing a baseline reference for comparison. The term αi refers to the effect of egg storage duration, where *i* is either one of the two levels (14 or 21 days). β_j represents the effect of preincubation thermal conditioning (PTC), where *j* is either one of the two levels (no-PTC or 6-hour PTC). The interaction term, $(\alpha \times \beta)_{ij}$ captures how egg storage duration and PTC interact. Finally, ε_{ijk} captures the residual error term, which accounts for the variability in the data that could not be explained by the fixed effects (storage duration, PTC, or their interaction). Furthermore, Pearson correlation analysis was employed to examine relationships between incubation duration and key physiological parameters such as rectal temperature, hematology, and serum metabolites, with statistical significance set at $p < 0.05$.

RESULTS AND DISCUSSION

Effect of prolonged storage and pre-incubation thermal conditioning on egg quality

Table 1 presents the effects of prolonged egg storage and pre-incubation thermal conditioning (PTC) on basic egg quality parameters after storage. The results indicate no significant differences in final egg weight, egg weight loss, yolk weight, shell weight, albumen weight, or shell thickness ($p > 0.05$), suggesting that neither prolonged storage nor PTC significantly influenced these attributes. This finding contrasts with the studies by Fasenko et al. (2001), Addo et al. (2018), and Abioja et al. (2021), which reported that prolonged storage adversely affects these quality parameters. However, as illustrated in Figure 2, the Haugh unit (HU), a critical indicator of egg protein quality, confirms that prolonged storage duration negatively impacted egg quality. Specifically, eggs stored for both 14 and 21 days showed a significant decline in protein quality, with HU values of 68 and 49 compared to 81 and 78 for eggs stored for shorter durations of 3 and 7 days, respectively ($p < 0.05$). These results are consistent with previous research, which demonstrates that prolonged storage negatively impacts egg protein quality (Scott and Silversides, 2001; Sekeroglu et al., 2008; Akyurek and Okur, 2009; Chung and Lee, 2014; Adeoye et al., 2023). A key finding in this study is that pre-incubation thermal conditioning (PTC) helped mitigate the deterioration of protein quality caused by prolonged storage. For eggs stored for 14 days, PTC significantly improved the Haugh Unit (HU) to 72, compared to 68 in the non-PTC control group ($p < 0.05$).

This effect was even more pronounced in eggs stored for 21 days, where PTC-treated eggs maintained an HU of 65, while the control group dropped to 49 ($p < 0.05$). Furthermore, PTC significantly increased blastoderm diameter, a critical marker of early embryogenesis compared to the control groups ($p < 0.05$), confirming the findings that PTC not only preserves egg protein integrity but also enhances early embryogenesis during extended storage (Romão et al., 2008).

Table 1. Effects of prolonged storage and pre-incubation thermal conditioning on egg quality after storage in Plymouth rock hybrid chickens

Treatment	IEW (g)	FEW (g)	% EWL	WY-Wt (g)	DY-Wt (g)	WS-Wt (g)	DS-Wt (g)	ALB-Wt (g)	WST (cm)	DST (cm)	BD (cm)
non-PTC×3d	55.70	55.50	9.15	26.30	25.1	13.1	10.58	63.60	0.57	0.55	0.31^{c}
non-PTC×7d	55.40	55.20	9.17	27.60	26.4	13.8	10.30	62.50	0.55	0.50	0.32^{c}
non-PTC×14d	55.10	49.70	9.80	23.70	23.6	11.8	10.20	62.30	0.53	0.45	0.26^{d}
6hr-PTC×14d	55.10	50.00	9.25	24.10	24.5	11.5	10.10	62.30	0.54	0.47	0.36^{b}
non-PTC×21d	55.10	49.90	9.45	23.90	23.6	12.0	10.40	61.00	0.50	0.42	0.27^{d}
6hr-PTC-21d	55.10	50.20	9.00	24.90	24.6	12.6	10.40	61.80	0.52	0.44	0.41^{a}
SEM	0.321	0.732	1.27	0.430	0.421	0.220	0.330	0.523	0.432	0.221	0.002
P-value	0.624	0.124	0.637	0.157	0.157	0.157	0.129	0.221	0.070	0.060	<0.001

Means of different superscripts ($^{a, b, c, d}$) within a column differ significantly at $p < 0.05$. SD: Storage duration; PTC: 6-hour preincubation thermal conditioning; non-PTC×3d: Fresh eggs stored for 3 days without PTC (control), non-PTC×7d: Fresh eggs stored for 7 days without PTC (control), non-PTC×14d: Fresh eggs stored for 14 days without PTC (control), 6hr-PTC×14d: Fresh eggs stored for 14 days with PTC non-PTC×21d: Fresh eggs stored for 21 days without PTC (control), 6hr-PTC-21d: Fresh eggs stored for 21 days with PTC, EW: Initial egg weight (g), FEW: Final egg weight (g), %EWL: Egg weight loss, WY-Wt: Wet yolk weight (g), DY-Wt: Dry yolk weight (g), WS-Wt: Wet shell weight (g), DS-Wt: Dry shell weight (g), ALB-Wt: Albumen weight (g), WST: Wet shell thickness (cm), DST: Dry shell thickness (cm), BD: Blastoderm diameter (cm). SEM: Pooled standard error of means. P-value: probability value.

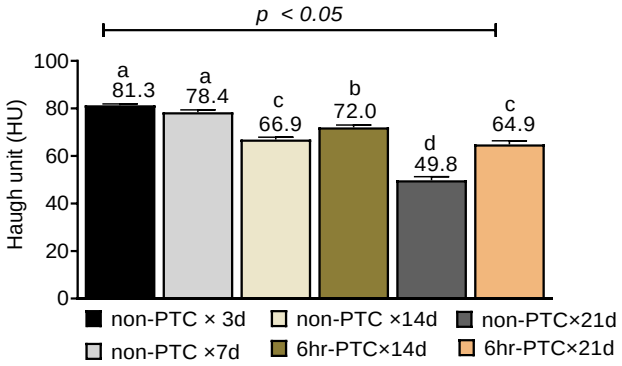

Figure 2. Effects of prolonged storage and pre-incubation thermal conditioning on Haugh unit as a measure of egg protein quality in Plymouth Rock hybrid chickens. non-PTC × 3d: Fresh eggs stored for 3 days without PTC (control), non-PTC×7d: Fresh eggs stored for 7 days without PTC (control), non-PTC×14d: Fresh eggs stored for 14 days without PTC (control), 6hr-PTC×14d: Fresh eggs stored for 14 days with 6hr-PTC, non-PTC×21d: Fresh eggs stored for 21 days without PTC (control), 6hr-PTC×21d: Fresh eggs stored for 21 days with PTC.

Effect of prolonged storage and pre-incubation thermal conditioning on embryo metabolic heat production during incubation

Table 2 shows the impact of storage duration and pre-incubation thermal conditioning (PTC) on the average eggshell temperature (AEST), which reflects the net metabolic heat production of embryos from incubation day 1 to 18 (ID1-18). No significant differences in AEST were observed between eggs stored for 14 and 21 days ($p > 0.05$). However, eggs subjected to PTC exhibited significantly higher AEST compared to the non-PTC (control) group for both storage durations ($p < 0.05$). As illustrated in Figure 3, the daily eggshell temperature (DEST) trends demonstrate that eggs stored for 14 days and treated with PTC maintained a more stable temperature, peaking at 38.5°C by ID18, compared to a fluctuating peak of 38.3°C in the non-PTC group. A similar pattern was observed in eggs stored for 21 days, where PTC-treated eggs reached a peak DEST of 38.6°C, while the control group exhibited a less stable rise, peaking at 38.0°C. These stable and higher DEST patterns in PTC-treated embryos suggest improved metabolic efficiency, as eggshell temperature serves as a key indicator of embryonic metabolic activity (Agyekum et al., 2022). Moreover, PTC may have activated pro-survival mechanisms, such as the upregulation of heat shock protein 70 (Hsp70), which is known to enhance embryonic metabolic activity and promote stress resilience (Jiang et al., 2011; Gan et al., 2015; Brady et al., 2022). This upregulation could explain the more stable and relatively higher eggshell temperatures observed in thermally conditioned embryos.

Table 2. Effects of prolonged storage and pre-incubation thermal conditioning on average eggshell temperature during incubation in Plymouth rock hybrid chickens

Factor	Eggshell temperature (°C)
SD	
14	37.94
21	37.89
SEM	0.057
P-value	0.536
PTC	
non-PTC	37.81[b]
6hr-PTC	38.02[a]
SEM	0.057
P-value	0.009
PTC × SD	
non-PTC × 14d	37.87
6hr-PTC × 14d	38.01
non-PTC × 21d	37.74
6hr-PTC × 21d	38.03
SEM	0.080
P-value	0.372

Means of different superscripts ([a, b]) within a column differ significantly at $p < 0.05$. SD: Storage duration; PTC: 6-hour preincubation thermal conditioning, non-PTC: Eggs that did not receive any PTC (control); 6hr-PTC: Eggs that received 6-hour PTC, PTC × SD: Non-PTC × 14d: Eggs stored for 14 days and did not receive PTC (control), 6hr-PTC × 14d: Eggs stored for 14 days and received PTC for 6 hours, non-PTC ×21d: Eggs stored for 21 days and did not receive PTC (control), 6hr-PTC × 21d: Eggs stored for 21 days and received PTC for 6 hours. SEM: Pooled standard error of means. P-value: Probability value.

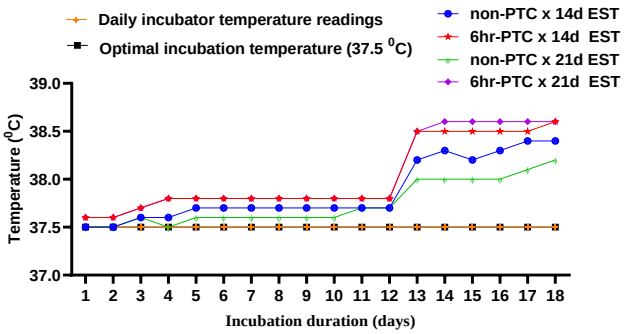

Figure 3. The pattern of daily eggshell temperature (DEST) and daily incubation temperature measured during the incubation period for Plymouth Rock Hybrid Chickens. The data points represent the average temperature measurements pooled from two separate incubators. EST: Eggshell temperature, non-PTC × 14d: Eggs stored for 14 days and did not receive PTC (control), 6hr-PTC × 14d: Eggs stored for 14 days and received PTC for 6 hours, non-PTC × 21d: Eggs stored for 21 days and did not receive PTC (control), 6hr-PTC × 21d: Eggs stored for 21 days and received PTC for 6 hours.

Effect of prolonged storage and pre-incubation thermal conditioning on relative egg weight loss during incubation

Table 3 presents the impact of storage duration and PTC on relative egg weight loss (REWL) during incubation. Extended storage increased REWL significantly, with eggs stored for 21 days losing 5.8% of their weight, compared to only 2.8% for those stored for 14 days ($p < 0.05$), as noted by Ruiz and Lunam (2002) and Khan et al. (2013). Pre-incubation thermal conditioning effectively reduced REWL across both storage periods. Specifically, PTC-treated eggs stored for 14 days lost only 4.0% of their weight compared to 5.6% in the control group. For eggs stored for 21 days, PTC-treated eggs showed an even lower weight loss of 1.5% while the control group lost 8.5% ($p < 0.05$). The increased REWL in the control group is likely due to albumen degradation and water loss (Hamidu et al., 2010). Overall, PTC demonstrated greater effectiveness in reducing weight loss, especially with prolonged storage, consistent with findings from Areaaer and Ibrahim (2019), Ansah et al. (2023), and Okasha et al. (2023).

Effect of prolonged storage and pre-incubation thermal conditioning on embryo growth dynamics during incubation

Table 4 shows the effects of prolonged storage and PTC on embryo growth. The results indicate that embryos from eggs stored for 14 days had significantly higher body weights by incubation day 18 (ID18) compared to those from the eggs stored for 21 days ($p < 0.05$). Notably, the application of PTC mitigated this negative impact and enhanced embryo growth compared to the non-PTC control group ($p < 0.05$). For eggs stored for 14 days, PTC-treated embryos had significantly higher body measurements by ID18, with wet weights of 23.1 g, dry weights of 8.2 g, and body lengths of 82.3 cm. In contrast, the control group recorded 22.7 g, 8.1 g, and 80.8 cm, respectively ($p < 0.05$). Similarly, for eggs stored for 21 days, PTC-treated embryos achieved 24.2 g wet weight, 9.7 g dry weight, and 97.3 cm in length, compared to 20.7 g, 7.2 g, and 71.8 cm for the control group ($p < 0.05$). These results affirm PTC's ability to mitigate the negative effects of prolonged storage, consistent with findings by Elmenawey (2019). Furthermore, Bakst et al. (2016) and Hemida et al. (2023) suggest that PTC upregulates heat shock protein 70 (Hsp70), enhancing metabolic processes and stress resilience while regulating anti-apoptotic proteins like B-cell lymphoma 2 (Bcl-2). This regulation likely inhibited early programmed cell death during prolonged storage, promoting cell survival and functionality, and ensuring effective cellular proliferation and differentiation, accounting for better growth outcomes observed in the PTC embryos (Kong et al., 2016).

Effect of prolonged storage and pre-incubation thermal conditioning on hatch outcomes

Figure 4 illustrates the effects of prolonged storage and PTC on hatch outcomes. Fertility rates remained consistent across all treatment groups, indicating that neither prolonged storage nor PTC adversely affected egg fertility. However, hatchability declined with longer storage periods, supporting findings from Ruiz and Lunam (2002), Mahmud et al. (2011), and Khan et al. (2013). Notably, PTC-treated eggs stored for 14 days achieved the highest hatchability rate of 76.4%, with a corresponding mortality rate of 23.6% and an incubation duration of 492 hours. In contrast, the non-PTC control group showed a hatchability rate of 65%, a mortality rate of 35%, and a longer incubation duration of 518.4 hours. For eggs stored for 21 days, PTC-treated embryos had a hatchability of 72.1%, with a mortality rate of 27.9% and an incubation duration of 496.8 hours, compared to a hatchability of 61.3%, a mortality rate of 38.7%, and an incubation duration of 530.4 hours in the control group. Overall, PTC improved hatchability by 11.4% and reduced incubation time by 26.4 hours for eggs stored for 14 days, increased hatchability by 10.8%, and reduced incubation duration by 33.6 hours for eggs stored for 21 days. These findings align with previous studies (Nicholson et al., 2013; Al-Samrai and Al-Dhanki, 2017; Areaaer and Ibrahim, 2019; Özlü et al., 2021; Abdel-Fattah et al., 2024), which emphasize PTC's role in enhancing hatchability and reducing incubation time. A shorter incubation duration is particularly beneficial, as prolonged incubation has been linked to reduced yolk sac absorption at the late stage of incubation (Özlü et al., 2021).

Effect of prolonged storage and pre-incubation thermal conditioning on post-hatch chick quality

Table 5 presents the impact of prolonged storage and PTC on post-hatch chick quality. Chicks hatched from eggs stored for 14 days had superior quality compared to those stored for 21 days ($p < 0.05$). The application of PTC significantly improved chick quality attributes compared to the control group ($p < 0.05$). For example, PTC-treated chicks from 14-day storage weighed 62.7 g, measured 146 mm in length, had a shank length of 27.3 mm, a residual yolk sac of 0.34 g, and a PASCAR score of 94.3%. In contrast, control chicks weighed 60.0 g, measured 115 mm, had a shank length of 25.9 mm, a residual yolk sac of 0.57 g, and a PASCAR score of 92.2%

($p < 0.05$). Similarly, for eggs stored for 21 days, PTC-treated chicks weighed 69.1 g, measured 138 mm, had a shank length of 22.2 mm, a residual yolk sac of 0.25 g, and a PASCAR score of 95.0%. The control group had significantly lower values: 47.5 g, 127 mm, 31.1 mm, 0.67 g, and a PASCAR score of 79.4% ($p < 0.05$). The improvements in body weight, PASCAR scores, and reduced residual yolk sac in PTC chicks support the findings of El-Garhy (2021) and Maman and Yildirim (2022). The reduced residual yolk sac in PTC-treated chicks indicates that PTC enhances yolk sac absorption, aligning with findings by Ebeid et al. (2017) and Damaziak et al. (2018) but contrasting those of Ansah et al. (2023). This improvement is crucial as embryos primarily depend on lipids from the yolk sac for energy during the final stages of incubation (Khosravinia, 2015).

Effect of prolonged storage and pre-incubation thermal conditioning on post-hatch chick thermoregulation capacity

Table 6 summarises the effects of prolonged egg storage and pre-incubation thermal conditioning (PTC) on chick rectal temperature (CRT) within the first 24 hours post-hatching. Chicks hatched from eggs stored for 21 days exhibited significantly higher CRTs compared to those stored for 14 days ($p < 0.05$), indicating a heightened metabolic rate and potential stress from compromised heat dissipation (Maman et al., 2019). However, PTC was effective in reducing CRT for both storage durations, helping maintain body temperature within a stable range. For instance, chicks from 14-day stored eggs with PTC showed a CRT of around 40.0°C, aligning with normal thermoregulation, whereas non-PTC counterparts recorded a higher CRT of 41.58°C ($p < 0.05$). Similarly, for 21-day stored eggs, PTC-treated chicks displayed a CRT of 40.07°C, notably lower than the 42.25°C observed in non-PTC chicks ($p < 0.05$). Figure 5 complements this by showing the CRT patterns across the 24 hours post-hatching, where PTC-treated chicks exhibited more stable and moderate temperatures. This stabilization suggests that PTC may enhance thermoregulatory capacity, potentially by supporting the development of key hormonal pathways, such as the hypothalamic-pituitary-thyroid and hypothalamic-pituitary-adrenal axes, which are essential for thermoregulation in homoeothermic animals (Debonne et al., 2008). These hormonal adaptations are likely to improve metabolic efficiency, allowing chicks to better regulate body temperature, an advantageous trait, especially for tropical breeds like Plymouth Rock (Ouchi et al., 2022). These findings align with previous studies (Nilsson and Persson, 2004; Page et al., 2022; El-Prollosy et al., 2024) suggesting that pre-incubation thermal conditioning can positively influence post-hatch thermoregulation and mitigate metabolic stress, especially for chicks hatched from eggs stored for extended periods. In contrast, the higher and more variable CRT in non-PTC chicks implies a less developed thermoregulatory response, potentially making them more susceptible to heat stress and dehydration, factors that could elevate early mortality risks (Romijn, 1954; Dunnington and Siegel, 1984; Decuypere et al., 2001).

Effect of prolonged storage and pre-incubation thermal conditioning on chick haematology

Table 7 highlights the effects of prolonged storage and PTC treatment on chick hematology, revealing that prolonged storage significantly impacted key parameters ($p < 0.05$). For eggs stored for 14 days, the non-PTC group exhibited a red blood cell (RBC) count of 2.10×10^{12}/L, significantly lower ($p < 0.05$) than the normal range of $2.2–4.0 \times 10^{12}$/L (Joshua et al., 2022), indicating reduced oxygen-carrying capacity. In contrast, the PTC group had a count of 2.37×10^{12}/L, which is within the normal range. The hemoglobin (HGB) level for the non-PTC group was 6.08 g/dL, significantly lower ($p < 0.05$) than the normal range of 7.0–11.0 g/dL (Benjamin, 1985), reflecting a potential risk of anemia, while the PTC group had a higher HGB of 8.67 g/dL, within normal limits. Likewise, for eggs stored for 21 days, the non-PTC group showed a significantly reduced RBC count of 1.65×10^{12}/L and HGB of 7.66 g/dL, both significantly lower ($p < 0.05$) than normal ranges, indicating severe anemia. Conversely, the PTC group maintained levels of 2.21×10^{12}/L and 9.20 g/dL, respectively, both within normal ranges (Merck veterinary manual, 2023). Haematocrit (HCT) also followed this trend, with the PTC group showing significantly higher values (32.9% versus 27.8% for non-PTC), indicating stable blood volume and improved health. Additionally, the PTC group had significantly higher mean corpuscular volume (136 versus134 fL), mean corpuscular hemoglobin (29.9 versus 28.7 pg), and mean corpuscular hemoglobin concentration (255 versus 247 g/dL, $p < 0.05$), reflecting enhanced blood oxygenation. Platelet counts were within normal ranges (Joshua et al., 2022) across all treatments, with the PTC group showing numerically higher counts, indicating better clotting ability. Overall, PTC treatment effectively mitigates the adverse effects of prolonged storage, promoting optimal growth and reducing the risk of anemia in Plymouth rock hybrid chickens.

Table 3. Effects of prolonged storage and pre-incubation thermal conditioning on relative egg weight loss during incubation in Plymouth rock hybrid chickens

Factor	Initial egg weight (g)	Final egg weight (g)	Relative egg weight loss (%)
SD			
14	50.14	48.75a	2.77[b]
21	50.21	47.29b	5.82[a]
SEM	0.032	0.730	1.270
P-value	0.066	0.017	0.029
PTC			
non-PTC	50.37	47.18[b]	6.33[a]
6hr-PTC	50.44	48.85[a]	3.15[b]
SEM	0.032	0.730	1.270
P-value	0.054	0.012	0.043
PTC × SD			
non-PTC × 14d	50.69	46.39[c]	5.55[b]
6hr-PTC × 14d	50.21	48.19[ab]	4.02[b]
non-PTC × 21d	50.80	47.98[b]	8.48[a]
6hr-PTC × 21d	50.26	49.52[a]	1.47[c]
SEM	0.045	1.030	1.800
P-value	0.090	< 0.001	< 0.001

Means of different superscripts ([a, b, c]) within a column differ significantly at $p < 0.05$. SD: Storage duration; PTC: 6-hour preincubation thermal conditioning, non-PTC: Eggs that did not receive any PTC (control); 6hr-PTC: Eggs that received 6-hour PTC, PTC × SD: non-PTC × 14d: Eggs stored for 14 days and did not receive PTC (control), 6hr-PTC × 14d: Eggs stored for 14 days and received PTC for 6 hours, non-PTC ×21d: Eggs stored for 21 days and did not receive PTC (control), 6hr-PTC × 21d: Eggs stored for 21 days and received PTC for 6 hours. SEM: Pooled standard error of means. P-value: Probability value.

Table 4. Effects of prolonged storage and pre-incubation thermal conditioning on embryo growth during incubation in Plymouth rock hybrid chickens

	Wet embryo weight (g)					Dry embryo weight (g)					Embryo length (cm)				
Factor	**4**	**7**	**11**	**14**	**18**	**4**	**7**	**11**	**14**	**18**	**4**	**7**	**11**	**14**	**18**
SD															
14	0.19^{a}	0.94^{a}	4.21^{a}	7.98^{a}	24.40^{a}	0.065	0.26^{a}	2.16^{a}	7.68^{a}	10.16^{a}	19.8^{a}	26.8^{a}	42.1^{a}	79.8^{a}	84.6^{a}
21	0.18^{b}	0. 69^{b}	3.62^{b}	6.96^{b}	22.20^{b}	0.080	0.20^{b}	2.01^{b}	6.48^{b}	8.14^{b}	18.1^{b}	20.3^{b}	36.2^{b}	69.6^{b}	81.6^{b}
SEM	0.002	0.021	0.021	0.006	0.510	0.012	0.003	0.040	0.091	0.350	0.580	0.320	1.55	0.870	2.54
P-value	0.009	< 0.001	<0.001	< 0.001	0.017	0.374	< 0.001	0.018	< 0.001	0.636	0.048	< 0.001	0.016	< 0.001	0.041
PTC															
non-PTC	0.19	0.20^{b}	3.24^{b}	6.91^{b}	21.7^{b}	0.057	0.57^{b}	1.78^{b}	5.50^{b}	5.63^{b}	18.1	20.1^{b}	32.4^{b}	69.1^{b}	76.3^{b}
6hr-PTC	0.18	0.27^{a}	4.58^{a}	8.03^{a}	23.2^{a}	0.088	1.10^{a}	2.39^{a}	6.66^{a}	8.98^{a}	19.8	27.1^{a}	45.8^{a}	80.3^{a}	89.8^{a}
SEM	0.002	0.003	0.021	0.006	0.510	0.012	0.021	0.040	0.091	0.35	0.58	0.32	1.55	0.87	2.54
P-value	0.940	< 0.001	< 0.001	<0.001	0.040	0.089	< 0.001	< 0.001	<0.001	0.020	0.763	< 0.001	< 0.001	< 0.001	0.002
PTC× SD															
non-PTC × 14d	1.93	0.158^{c}	3.88^{a}	6.28^{c}	22.72^{b}	0.040	0.41^{d}	2.03^{b}	4.62^{d}	8.08^{b}	18.3	25.3^{b}	26.1^{b}	76.3^{b}	80.8^{b}
6hr-PTC × 14d	2.03	0.26^{b}	4.50^{a}	8.33	23.10^{ab}	0.090	0.97^{b}	2.29^{a}	7.83^{b}	8.23^{b}	19.8	25.8^{b}	45.3^{a}	77.3^{b}	82.3^{b}
non-PTC × 21d	1.88	0.25^{b}	2.61^{b}	7.53^{b}	20.7^{b}	0.075	0.74^{c}	1.52^{c}	6.37^{c}	7.18^{b}	17.3	14.8^{c}	38.8^{b}	62.8^{c}	71.8^{b}
6hr-PTC × 21d	1.73	0.28^{a}	4.63^{a}	8.43^{a}	24.2^{a}	0.085	1.15^{a}	2.50^{a}	9.00^{a}	9.73^{a}	20.3	28.3^{a}	46.3^{a}	84.3^{a}	97.3^{a}
SEM	0.003	0.004	0.031	0.009	0.720	0.017	0.030	0.056	0.130	0.490	0.820	0.450	2.19	1.23	3.59
P-value	0.612	0.028	0.292	< 0.001	0.010	0.243	0.023	< 0.001	0.001	0.036	0.145	< 0.001	0.006	0.036	0.004

Means of different superscripts ($^{a, b, c}$) within a column differ significantly at $p < 0.05$. SD: Storage duration; PTC: 6-hour preincubation thermal conditioning, non-PTC: Eggs that did not receive any PTC (control); 6hr-PTC: Eggs that received 6-hour PTC, PTC × SD: Non-PTC × 14d: Eggs stored for 14 days and did not receive PTC (control), 6hr-PTC × 14d: Eggs stored for 14 days and received PTC for 6 hours, non-PTC ×21d: Eggs stored for 21 days and did not receive PTC (control), 6hr-PTC × 21d: Eggs stored for 21 days and received PTC for 6 hours. SEM: Pooled standard error of means. P-value: Probability value.

Table 5. Effects of prolonged storage and pre-incubation thermal conditioning on post-hatch chick quality in Plymouth Rock hybrid chickens

	Chick weight (g)		Chick length (mm)		Shank length (mm)		Residual yolk (g)		PASCAR
Factor	**Day 1**	**Day 7**	**Day 1**	**Day 7**	**Day 1**	**Day 7**	**Day 1**	**Day 7**	**Score (%)**
SD									
14	42.4^{a}	61.3^{a}	114^{a}	132^{a}	24.2^{a}	28.6^{a}	3.62^{b}	0.30^{b}	94.1^{a}
21	38.5^{b}	58.3^{b}	109^{b}	130^{b}	22.0^{b}	26.6^{b}	5.62^{a}	0.70^{a}	84.7^{b}
SEM	0.660	0.940	0.990	1.350	1.300	1.490	0.190	0.024	1.570
P-value	< 0.001	0.027	0.006	0.031	0.023	0.045	0.001	0.001	<0.001
PTC									
non-PTC	36.4^{b}	53.7^{b}	104^{b}	121^{b}	25.5^{a}	29.5^{a}	6.12^{a}	0.77^{a}	86.8^{b}
6hr-PTC	44.5^{a}	65.9^{a}	119^{a}	142^{a}	20.8^{b}	24.7^{b}	5.12^{b}	0.64^{b}	92.0^{a}
SEM	0.660	0.940	0.990	1.35	1.30	1.49	0.190	0.024	1.57
P-value	< 0.001	< 0.001	< 0.001	< 0.001	0.015	0.030	0.001	0.001	0.024
PTC × SD									
non-PTC × 14d	40.0^{b}	60.0^{b}	104^{c}	115^{d}	25.4^{a}	27.9^{ab}	6.12	0.77	92.2^{b}
6hr-PTC × 14d	44.8^{a}	62.7^{b}	123^{a}	146^{a}	23.0a^{b}	27.3^{ab}	5.12	0.64	94.3^{a}
non-PTC × 21d	32.7^{c}	47.5^{c}	104^{c}	127^{c}	25.5^{a}	31.1^{a}	6.12	0.77	79.4^{c}
6hr-PTC × 21d	44.3^{a}	69.1^{a}	115^{b}	138^{b}	18.5^{b}	22.2^{b}	5.12	0.64	95.0^{a}
SEM	0.930	1.330	1.400	1.910	1.840	2.110	0.270	0.033	2.210
P-value	0.001	< 0.0001	0.008	< 0.0001	0.021	0.041	1.000	1.000	0.020

Means of different superscripts ($^{a, b, c, d}$) within a column differ significantly at $p < 0.05$. SD: Storage duration; PTC: 6-hour preincubation thermal conditioning, non-PTC: Eggs that did not receive any PTC (control); 6hr-PTC: Eggs that received 6-hour PTC, PTC × SD: non-PTC × 14d: Eggs stored for 14 days and did not receive PTC (control), 6hr-PTC × 14d: Eggs stored for 14 days and received PTC for 6 hours, non-PTC × 21d: Eggs stored for 21 days and did not receive PTC (control), 6hr-PTC × 21d: Eggs stored for 21 days and received PTC for 6 hours. SEM: Pooled standard error of means. P-value: Probability value.

Table 6. Effects of prolonged storage and pre-incubation thermal conditioning on the average rectal temperature of Plymouth Rock hybrid chickens measured hourly within 24 hours after hatching

Factor		Chick rectal temperature (^{0}C)
SD	14	40.64[b]
	21	41.16[a]
	SEM	0.260
	P-value	0.033
PTC	non-PTC	41.92[a]
	6hr-PTC	39.88[b]
	SEM	0.261
	P-value	0.013
PTC × SD	non-PTC × 14d	41.58[a]
	6hr-PTC × 14d	39.69[b]
	non-PTC × 21d	42.25[a]
	6hr-PTC × 21d	40.07[b]
	SEM	0.368
	P-value	0.045

Means of different superscripts ([a, b]) within the column differ significantly at $p < 0.05$. SD: Storage duration; PTC: 6-hour preincubation thermal conditioning, non-PTC: Eggs that did not receive any PTC (control); 6hr-PTC: Eggs that received 6-hour PTC, PTC × SD: Non-PTC × 14d: Eggs stored for 14 days and did not receive PTC (control), 6hr-PTC × 14d: Eggs stored for 14 days and received PTC for 6 hours, non-PTC ×21d: Eggs stored for 21 days and did not receive PTC (control), 6hr-PTC × 21d: Eggs stored for 21 days and received PTC for 6 hours. SEM: Pooled standard error of means. P-value: Probability value.

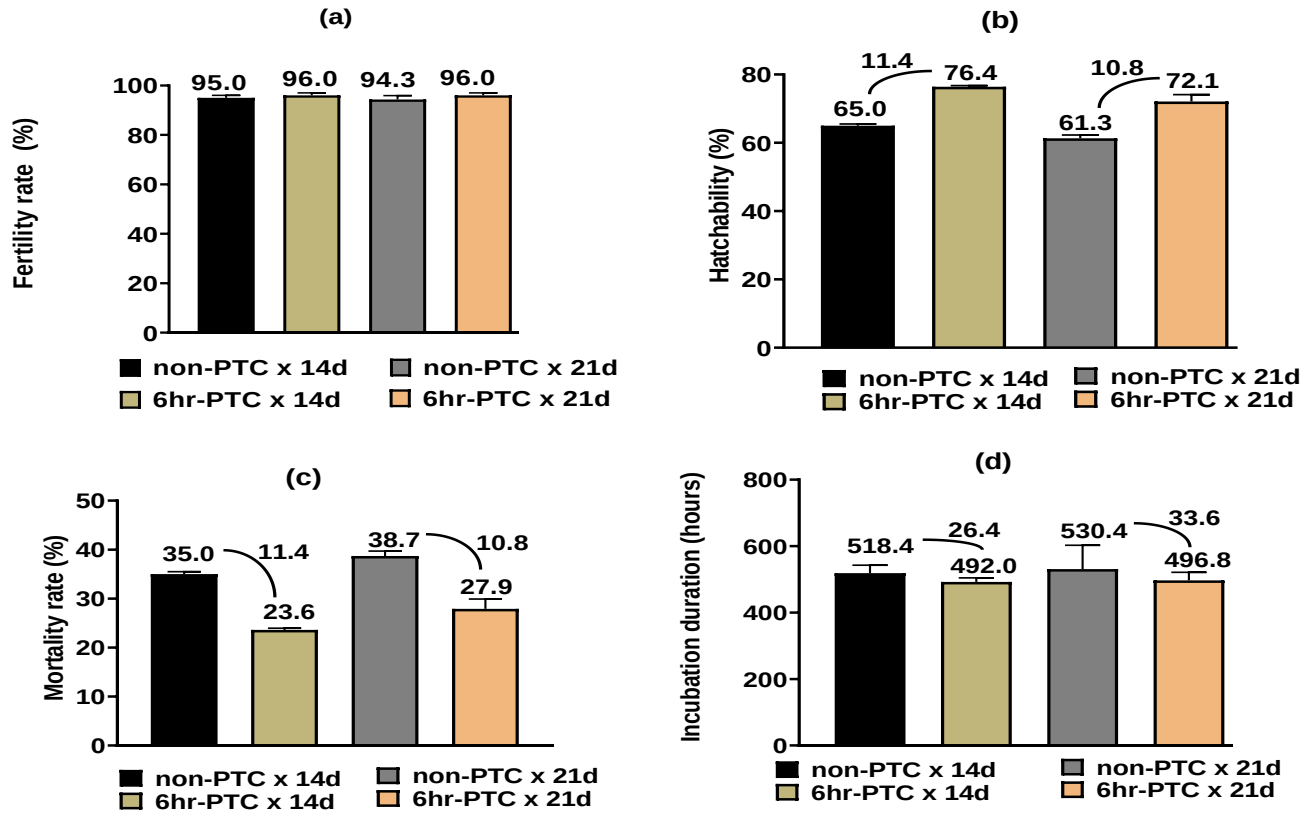

Figure 4. Effect of prolonged storage and pre-incubation thermal conditioning on fertility rate (a), hatchability rate (b), mortality rate (c), and incubation duration (d) in Plymouth Rock Hybrid chickens. Data bars are average values pooled from the two separate incubators. non-PTC × 14d: Eggs stored for 14 days and did not receive PTC (control), 6hr-PTC × 14d: Eggs stored for 14 days and received PTC for 6 hours, non-PTC × 21d: Eggs stored for 21 days and did not receive PTC (control), 6hr-PTC × 21d: Eggs stored for 21 days and received PTC for 6 hours.

To cite this paper: Sasu P, Agbehadzi RK, Ackah EM, Adjei-Mensah B, Felicia EE, Danquah CA, Tona K, Hamidu JA, and Were P (2025). Effects of Six-Hour Pre-Incubation Thermal Conditioning and Prolonged Storage on Egg Quality, Embryogenesis, Hatchability, and Post-Hatch Physiology of Plymouth Rock Hybrid Chickens in Tropical Climate of Ghana. *J. World Poult. Res.*, 15(1): 1-22. DOI: https://dx.doi.org/10.36380/jwpr.2025.1

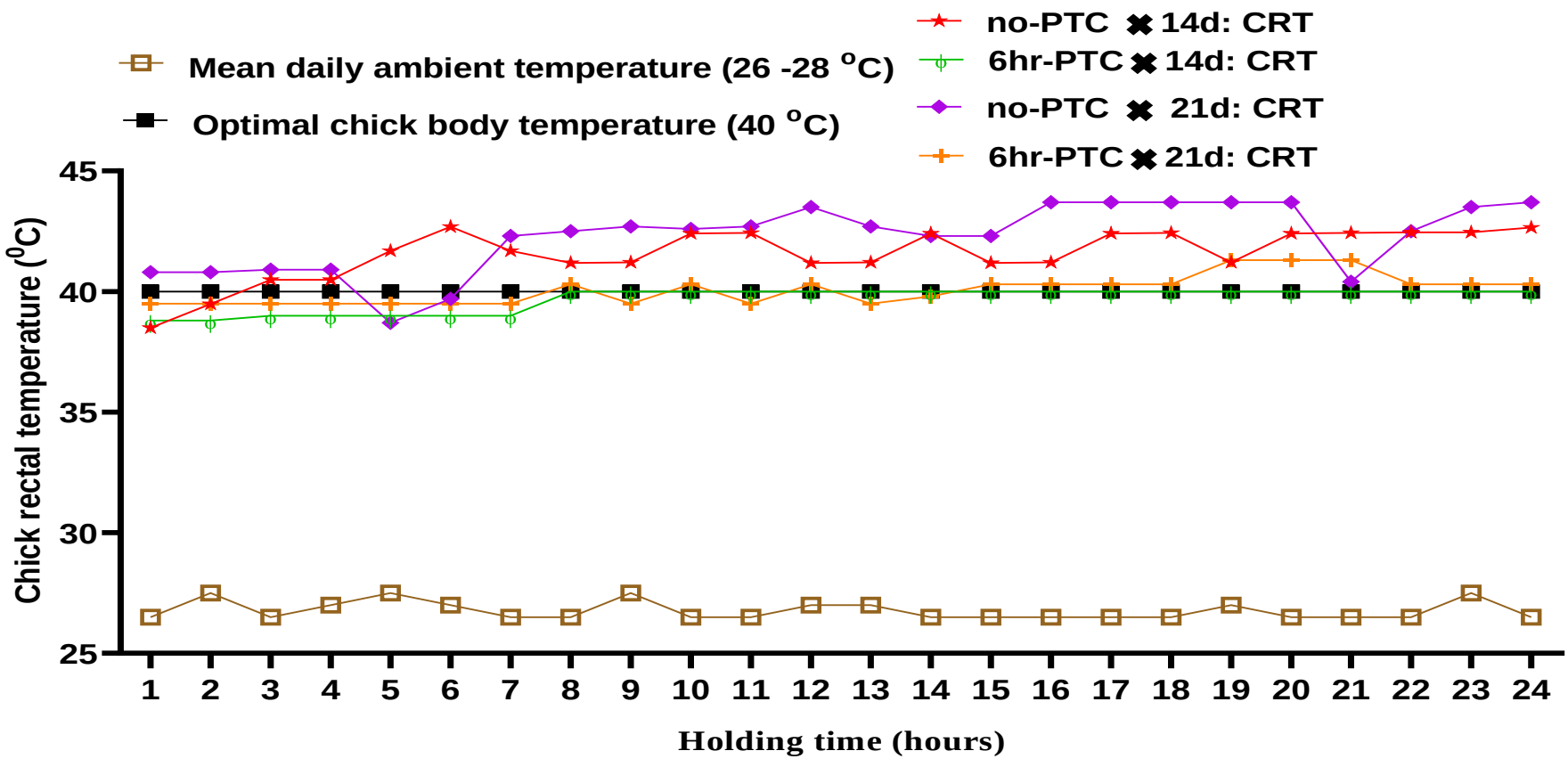

Figure 5. Effects of prolonged egg storage and pre-incubation thermal conditioning on patterns of rectal temperature of Plymouth Rock hybrid chickens measured within the first 24 hours post-hatching. CRT: Chick rectal temperature, non-PTC × 14d: Eggs stored for 14 days and did not receive PTC (control), 6hr-PTC × 14d: Eggs stored for 14 days and received PTC for 6 hours, non-PTC × 21d: Eggs stored for 21 days and did not receive PTC (control), 6hr-PTC × 21d: Eggs stored for 21 days and received PTC for 6 hours.

Table 7. Effects of prolonged storage and pre-incubation thermal conditioning on hematological profile in Plymouth Rock Hybrid chickens

	Erythrocytes						Platelets			
Factor	**RBC (10^{12}/L)**	**HGB (g/dL)**	**HCT/ PCV (%)**	**MCV (f/L)**	**MCH (pg)**	**MCHC (g/dL)**	**PLT (10^9/L)**	**MPV (fL)**	**PDW (%)**	**PCT (%)**
SD										
14	2.00	8.93^{a}	30.9^{a}	135^{a}	29.2	252^{a}	22.6^{a}	15.9^{a}	14.9	0.021
21	2.13	7.92^{b}	28.6^{b}	132^{b}	29.5	246^{b}	18.5^{b}	13.7^{b}	14.8	0.023
SEM	0.140	0.100	0.08	0.12	0.11	0.11	0.15	0.11	0.037	0.002
P-value	0.546	< 0.001	< 0.001	< 0.001	0.330	< 0.001	< 0.001	< 0.001	0.058	0.465
PTC										
non-PTC	2.21	6.42	29.1^{b}	132^{b}	29.3	248^{b}	19.8^{b}	14.2^{b}	14.5^{b}	0.016^{b}
6hr-PTC	1.93	8.03	30.3^{a}	135^{a}	29.3	251^{a}	21.2^{a}	15.4^{a}	15.2^{a}	0.027^{a}
SEM	0.140	0.100	0.08	0.12	0.11	0.11	0.15	0.11	0.037	0.003
P-value	0.181	0.973	< 0.001	< 0.001	0.841	< 0.001	< 0.001	< 0.001	<0.001	0.001
PTC × SD										
non-PTC × 14d	2.10	6.08^{bc}	28.9^{b}	130.0^{c}	28.9^{b}	246^{c}	17.8	13.2	14.6	0.015
6hr-PTC × 14d	2.37	8.67^{ab}	29.5^{b}	135.0^{a}	29.8^{a}	249^{b}	21.8	15.1	14.4	0.018
non-PTC × 21d	1.65	6.66^{b}	27.8^{c}	134.0^{b}	28.7^{b}	247^{c}	19.1	14.2	15.2	0.027
6hr-PTC × 21d	2.21	9.20^{a}	32.9^{a}	136.0^{a}	29.9^{a}	255^{a}	23.4	16.6	15.2	0.028
SEM	0.20	0.14	0.11	0.17	0.16	0.16	0.21	0.160	0.053	0.002
P-value	0.056	0.006	< 0.001	< 0.001	< 0.001	< 0.001	0.545	0.153	0.153	0.658

Means of different superscripts ($^{a, b, c}$) within a column differ significantly at $p < 0.05$. RBC (10^{12}/L): Red blood cell, HGB (g/dL): Haemoglobin, HCT/PCV: Haematocrit / Packed cell volume, MCV (f/L): Mean corpuscular volume, MCH (pg): Mean corpuscular haemoglobin, MCHC (g/dL): Mean corpuscular haemoglobin concentration, PLT (10^9/L): Platelet count, MPV: Mean platelet volume, PDW (%): Platelet distribution width, PCT (%): Plateletcrit. SD: Storage duration; PTC: 6-hour preincubation thermal conditioning, non-PTC: Eggs that did not receive any PTC (control); 6hr-PTC: Eggs that received 6-hour PTC, PTC × SD: non-PTC × 14d: Eggs stored for 14 days and did not receive PTC (control), 6hr-PTC × 14d: Eggs stored for 14 days and received PTC for 6 hours, non-PTC ×21d: Eggs stored for 21 days and did not receive PTC (control), 6hr-PTC × 21d: Eggs stored for 21 days and received PTC for 6 hours. SEM: Pooled standard error of means. P-value: Probability value.

Table 8. Effect of prolonged storage and pre-incubation thermal conditioning on serum metabolites in Plymouth Rock Hybrid Chickens

	Proteins			Carbohydrate	Lipids		Thyroid hormones	
Factor	**T-Pro (g/L)**	**GBL (g/L)**	**ALB (g/L)**	**Gluco (g/L)**	**T-Chol (g/L)**	**Trig (g/L)**	**T3 (pmol/L)**	**T4 (pmol/L)**
SD								
14	22.40	7.26^{a}	17.92^{a}	2.84	6.38	1.66	2.59^{b}	1.89^{b}
21	20.83	2.91^{b}	15.13^{b}	3.63	6.31	1.39	3.21^{a}	2.48^{a}
SEM	0.495	0.505	0.674	0.120	0.495	0.212	0.170	0.144
P-value	0.055	< 0.001	0.019	0.087	0.928	0.394	0.019	0.020
PTC								
non-PTC	18.93^{b}	4.33^{b}	14.60^{b}	3.84^{a}	6.67	1.81	3.95^{a}	3.03^{a}
6hr-PTC	24.29^{a}	5.84^{a}	18.45^{a}	2.63^{b}	6.02	1.25	1.86^{b}	1.34^{b}
SEM	0.495	0.505	0.674	0.120	0.495	0.212	0.170	0.144
P-value	< 0.001	0.048	0.004	0.018	0.384	0.099	<0.001	<0.001
PTC × SD								
non-PTC × 14d	18.83^{c}	5.95^{ab}	12.88^{c}	3.18^{ab}	6.70	1.30	3.60^{a}	3.62^{a}
6hr-PTC × 14d	25.96^{a}	8.58^{a}	17.38^{a}	2.49^{b}	6.05	2.02	1.58^{b}	1.34^{b}
non-PTC × 21d	19.03^{c}	2.71^{c}	16.32^{ab}	4.49^{a}	6.63	1.19	4.29^{a}	2.44^{a}
6hr-PTC × 21d	22.62^{b}	3.10^{bc}	19.52^{a}	2.77^{b}	5.99	1.59	2.13^{b}	1.35^{b}
SEM	0.701	0.715	0.953	0.170	0.264	0.300	0.241	0.204
P-value	0.035	0.036	0.014	0.024	0.701	0.608	0.045	0.020

Means of different superscripts ($^{a, b, c}$) within a column differ significantly at $p < 0.05$. T-Pro (g/L): Total protein, GBL (g/L): Globulin, ALB (g/L): Albumin, Gluco (g/L): Glucose, T-Cho (g/L): Total cholesterol, Trig (g/L): Triglyceride, T3 (pmol/L): Triiodothyronine, T4 (pmol/L): Thyroxine. SD: Storage duration; PTC: 6-hour preincubation thermal conditioning, non-PTC: Eggs that did not receive any PTC (control); 6hr-PTC: Eggs that received 6-hour PTC, PTC × SD: non-PTC × 14d: Eggs stored for 14 days and did not receive PTC (control), 6hr-PTC × 14d: Eggs stored for 14 days and received PTC for 6 hours, non-PTC ×21d: Eggs stored for 21 days and did not receive PTC (control), 6hr-PTC × 21d: Eggs stored for 21 days and received PTC for 6 hours. SEM: Pooled standard error of means. P-value: Probability value.

Table 9. Pairwise Pearson correlation analysis between incubation duration and key physiological parameters in Plymouth Rock Hybrid chickens

	Incubation duration (hours)	Chick rectal temperature (^{o}C)	HGB (g/dL)	HCT/ PCV (%)	MCV (f/L)	MCH (pg)	MCHC (g/dL)	Total protein (g/L)	Globulin (g/L)	Albumin (g/L)	Glucose (g/L)	T3 (pmol/L)
Chick rectal temperature (^{o}C)	0.854*											
HGB (g/dL)	-0.044	-0.788*										
HCT/ PCV (%)	-0.254	-0.498	0.855*									
MCV f/L	-0.000	-0.545	0.505	0.524								
MCH (pg)	0.055	-0.930*	0.729*	0.523	0.496							
MCHC (g/dL)	-0.185	-0.567	0.799*	0.950*	0.728*	0.600*						
Total protein (g/L)	-0.086	-0.819*	0.692*	0.394	0.673*	0.713*	0.482					
Globulin (g/L)	0.052	-0.607*	0.303	-0.161	-0.075	0.541*	-0.196	0.634*				
Albumin (g/L)	0.046	-0.700*	0.584*	0.623*	0.903*	0.723*	0.823*	0.623*	-0.012			
Glucose (g/L)	0.830*	0.093	-0.434	-0.450	0.010	-0.162	-0.304	-0.385	-0.264	0.036		
T3 (pmol/L)	0.683*	0.426	-0.621*	-0.560	-0.404	-0.457	-0.532*	-0.730*	-0.362	-0.358	0.886*	
T4 (pmol/L)	0.655*	0.173	-0.389	-0.562	-0.433	-0.247	-0.616	-0.427	0.11	-0.454	0.697*	0.794*

* Significant at $p < 0.05$. HGB (g/dL): Haemoglobin, HCT/PCV: Haematocrit/Packed cell volume, MCV (f/L): Mean corpuscular volume, MCH (pg): Mean corpuscular haemoglobin, MCHC (g/dL): Mean corpuscular haemoglobin concentration, T3 (pmol/L): Triiodothyronine, T4 (pmol/L): Thyroxine

Effect of prolonged storage and pre-incubation thermal conditioning on chick serum metabolites

Table 8 presents the effects of storage duration and PTC on chick serum metabolites. The findings show that storage duration significantly influenced several serum parameters, except for total protein (T-Pro), total cholesterol (T-Chol), glucose, and triglycerides (Trig, $p < 0.05$). Chicks hatched from eggs stored for 21 days had significantly lower globulin (GBL) and albumin (ALB) levels but higher triiodothyronine (T3) and thyroxine (T4) levels compared to chicks from eggs stored for 14 days ($p < 0.05$). However, PTC application significantly mitigated these effects, increasing total protein, globulin, and albumin levels while reducing glucose and triiodothyronine levels compared to the non-PTC group ($p < 0.05$). Specifically, eggs stored for 14 days and subjected to PTC resulted in chicks with significantly higher total protein (25.96 g/L), globulin (8.58 g/L), and albumin (17.38 g/L) levels, but lower glucose (2.49 g/L), triiodothyronine (1.58 pmol/L), and thyroxine (1.34 pmol/L) compared to eggs stored for 14 days without PTC, which had total protein (18.83 g/L), globulin (5.95 g/L), albumin (12.88 g/L), glucose (3.18 g/L), triiodothyronine (3.60 pmol/L), and thyroxine (3.62 pmol/L, $p < 0.05$). Similarly, eggs stored for 21 days and subjected to PTC produced chicks with significantly higher total protein (22.62 g/L), globulin (3.10 g/L), and albumin (19.52 g/L) levels but lower glucose (2.77 g/L), triiodothyronine (2.13 pmol/L), and thyroxine (1.35 pmol/L) compared to eggs stored for 21 days without PTC, which recorded total protein (19.03 g/L), globulin (2.71 g/L), albumin (16.32 g/L), glucose (4.49 g/L), triiodothyronine (4.29 pmol/L), and thyroxine (2.44 pmol/L, $p < 0.05$). No significant interaction effects were found for total cholesterol and triglycerides ($p > 0.05$). The lower plasma protein levels and elevated glucose concentrations in the non-PTC chicks suggest an increased metabolic rate as they may be adjusting to thermal stress (Tanizawa et al., 2014). Their heightened levels of thyroxine (T4) further suggest that the thyroid gland was more active, while the elevated triiodothyronine (T3) levels indicate intensified peripheral deiodination, both mechanisms aimed at coping with thermal challenges (Yahav et al., 2004). In contrast, PTC-treated chicks demonstrated lower levels of T3 and T4, indicating a reduced metabolic rate (Yahav and Hurwitz, 1996), likely as part of a key mechanism in thermotolerance acquisition, where heat production is suppressed by decreasing T3 concentrations (Yahav and McMurtry, 2001). These findings in conjunction with the improved haematological profiles and serum protein levels in the PTC-treated chicks suggest that PTC application not only improves metabolic regulation of chicks but also enhances their ability to withstand post-hatch thermal stress.

Pearson correlations between incubation duration and key physiological parameters

Table 9 provides crucial insights through the Pearson correlation analysis, shedding light on the physiological mechanisms behind the observed findings in the study. These correlations help explain the challenges faced by non-PTC chicks and how PTC application improved their physiological responses. A strong positive correlation between incubation duration and rectal temperature ($r = 0.854$, $p < 0.05$) supports the observation that non-PTC chicks, which underwent prolonged incubation, exhibited elevated rectal temperatures. This finding aligns with the idea that these chicks struggled with thermoregulation, as newly hatched chicks are dependent on their environment to maintain body temperature. The inability to dissipate heat effectively indicates that these chicks faced metabolic stress, further explaining the thermoregulatory challenges observed in non-PTC chicks. In contrast, the normal rectal temperatures observed in PTC-treated chicks can be attributed to the positive influence of PTC on their ability to manage thermal stress. The correlation suggests that PTC improved the chicks' thermotolerance by stimulating mechanisms within the sympathetic nervous system, which plays a critical role in temperature regulation post-hatch (Wekstein and Zolman, 1969). This perhaps enhanced thermoregulation and allowed PTC chicks to better cope with the environmental stressors they encountered after hatching. Furthermore, the negative correlations between elevated rectal temperatures and critical blood parameters, such as hemoglobin (HGB, $r = -0.788$, $p < 0.05$), mean corpuscular hemoglobin (MCH, $r = -0.930$, $p < 0.05$), and total protein ($r = -0.819$, $p < 0.05$), reinforce the earlier observation of dehydration and reduced oxygen-carrying capacity in non-PTC chicks. These correlations suggest that the increased rectal temperatures contributed to a breakdown in homeostasis, where dehydration likely impaired protein synthesis, leading to reduced plasma protein levels and poorer growth and development observed in these chicks (Xin and Rieger, 1995). Additionally, the positive correlation between incubation duration and glucose levels ($r = 0.830$, $p < 0.05$) helps explain why non-PTC chicks exhibited higher plasma glucose concentrations. This increase

reflects a stress-induced metabolic response, where the chicks needed to boost energy production to meet the demands of thermoregulation. This heightened glucose reliance underlines the increased metabolic strain experienced by these chicks in the absence of PTC. Finally, the significant correlations between triiodothyronine (T3, $r = 0.683$, $p < 0.05$) and glucose (T3: $r = 0.886$, T4: $r = 0.697$, $p < 0.05$) further explain the hormonal changes observed in non-PTC chicks. Elevated T3 and T4 levels are indicative of increased metabolic activity, as the chicks ramped up their energy production to compensate for thermal stress. These findings are consistent with previous studies showing that increased thyroid hormone activity is a physiological response to thermal challenges, enabling chicks to manage the energy demands of thermoregulation (Yahav and McMurtry, 2001).

CONCLUSION

The present study demonstrated that six-hour pre-incubation thermal conditioning (PTC) effectively mitigates the adverse impacts of prolonged egg storage in Plymouth rock hybrid chickens. Notably, the application preserved egg quality, enhanced embryonic development, restored hatchability, and improved post-hatch chick vitality and overall health, indicating its potential as a beneficial strategy in hatchery operations. However, the variability in PTC efficacy across the different storage durations necessitates further research to assess its broader applicability and economic feasibility in different poultry breeds, particularly in tropical climates.

DECLARATIONS

Availability of data and materials

The datasets generated and analysed during the current study are available from the corresponding author upon reasonable request.

Funding

The World Bank Group funded this research through the Regional Centre of Excellence in Poultry Science (CERSA) at the University of Lomé, Togo (Grant No. IDA 5424).

Authors' contributions

Prince Sasu conceptualized the study. Data collection was conducted by Prince Sasu, Felicia Emmanuella Ellison, Edna Mariam Ackah, Richard Koblah Agbehadzi, and Benjamin Adjei-Mensah. Data analysis and the initial manuscript draft were carried out by Prince Sasu and Benjamin Adjei-Mensah, with co-supervision provided by Cynthia Amaning Danquah, Kokou Tona, Jacob Alhassan Hamidu, and Were Pitala. All authors contributed to the editing and review of the manuscript and confirmed the last edition of the manuscript before submission to the journal.

Competing interests

The authors declare no conflicts of interest, either personal or professional, that could affect the interpretation of the findings presented in this manuscript.

Ethical considerations

All authors confirm adherence to ethical standards, including those concerning plagiarism, consent for publication, research misconduct, data fabrication, duplicate publication, and redundancy.

REFERENCES

Abdel-Fattah SA, Madkour M, Hemida MA, and Shourrap M (2024). Growth performance, histological and physiological responses of heat-stressed broilers in response to short periods of incubation during egg storage and thermal conditioning. Scientific Reports, 14(1): 94. DOI: https://www.doi.org/10.1038/s41598-023-50295

Abioja MO, Abiona JA, Akinjute OF, and Ojoawo HT (2021). Effect of storage duration on egg quality, embryo mortality and hatchability in FUNAAB-α chickens. Journal of Animal Physiology and Animal Nutrition, 105(4): 715-724. DOI: https://www.doi.org/10.1111/jpn.13480.

Addo A, Hamidu JA, Ansah AY, and Adomako K (2018). Impact of egg storage duration and temperature on egg quality, fertility, hatchability, and chick quality in naked neck chickens. International Journal of Poultry Science, 17(4): 175-183. DOI: https://www.doi.org/10.3923/ijps.2018.175.183

Adegbenro M, Omole TR, Jongbo AO, and Osho IB (2023). Influence of cage tiers height on cloaca temperature, performance, and egg quality of Isa-brown laying hens reared in a humid tropical climate. Journal of Archiva Zootechnica, 26(2): 116-134. DOI: https://www.doi.org/10.2478/azibna-2023-0018.

Adeoye A, Oyeleye O, Olorunsola R, Akinleye P, and Ojajuni J (2023). Storage duration impacts on nutritional composition and quality of table eggs. Bangladesh Journal of Animal Science, 52(1): 15-21. DOI: https://www.doi.org/10.3329/bjas.v52i1.65356

Adriaensen H, Parasote V, Castilla I, Bernardet N, Halgrain M, Lecompte F, and Réhault-Godbert S (2022). How egg storage duration before incubation impairs egg quality and chicken embryonic development: Contribution of imaging technologies. Frontiers in Physiology, 13: 902154. Available at: https://www.frontiersin.org/articles/10.3389/fphys.2022.902154/full

Agyekum G, Okai MA, Tona JK, Donkoh A, and Hamidu JA (2022). Impact of incubation temperature profile on chick quality, bone, and immune system during the late period of incubation of Cobb 500 broiler strain. Journal of Poultry Science, 101(9): 101999. DOI: https://www.doi.org/10.1016/j.psj.2022.101999

Akyurek H and Okur AA (2009). Effect of storage time, temperature and hen age on egg quality in free-range layer hens. Journal of Animal Veterinary Advances, 8(10): 1953-1958. Available at: https://www.cabidigitallibrary.org/doi/full/10.5555/20093222841

Alonge EO, Eruvbetine D, Idowu OMO, Obadina AO, and Olukomaiya OO (2017). Effect of dietary feed additives on haematological and serum biochemical parameters of broiler chickens. Online Journal of Animal and Feed Research, 7(1): 18-23. Available at: http://www.ojafr.com/main/attachments/article/125/

AL-Samrai EKE and Al-Dhanki ZTM (2017). Effect of pre-incubation in pre-storage and short periods of incubation during egg storage (SPIDES) and thermal manipulation during the incubation period in embryonic development and percentage of hatching characteristics of hatching eggs of broiler breeder (ROSS 308). Al-Anbar Journal of Veterinary Sciences, 15(2): 554-567. Available at: https://www.iasj.net/iasj/article/134933

Ansah SA, Ackah EM, Boateng M, Nurudeen L, Nyarko F, Acheampong KA, Sasu P, and Hamidu JA (2023). Impact of storage duration and short periods of incubation during egg storage on embryonic development and hatching traits of hybrid chicken strain. Journal of Animal Biotechnology, 34(8): 4081-4093. DOI: https://www.doi.org/10.1080/10495398

Areaaer AH and Ibrahim BM (2019). Effect of storage duration and heat treating of eggs hatching broiler breed Ross 308 before or during the period of storage in the characteristics of quality chicks hatched. Biochemical and Cellular Archives, 19(2): 3141-3146. DOI: https://www.doi.org/10.35124/bca.2019.19.2.3141

Bakst MR, Welch GR, Fetterer R, and Miska K (2016). Impact of broiler egg storage on the relative expression of selected blastoderm genes associated with apoptosis, oxidative stress, and fatty acid metabolism. Journal of Poultry Science, 95(6): 1411-1417. DOI: https://www.doi.org/10.3382/ps/pew038

Barde JI, Luka HS, Elisha IL, Nyam LS, Suleiman I, Gambo RA, and Samuel NS (2022). Haematological and serum biochemical parameters of broilers slaughtered at Bukuru live bird market of JOS south local government plateau state Nigeria. Acta Scientific Veterinary Sciences, 4(6): 5-11. DOI: https://www.doi.org/10.31080/asvs.2022.04.0399

Benjamin MM (1985). Outline of veterinary clinical pathology, 3rd Edtion. The Iowa State University Press., Ames, U.S.A., pp. 351-361. Available at: https://books.google.co.id/books/about/Outline_of_Veterinary_Clinical_Pathology.html?id=m11q9zqMe2EC&redir_esc=y

Bowers LD and Wong ET (1980). Kinetic serum creatinine assays. II. A critical evaluation and review. Clinical Chemistry, 26: 555-561. DOI: https://www.doi.org/10.1093/clinchem/26.5.0555

Brady K, Talbot CC, Long J, Welch GR, French NA, Nicholson DJ, and Bakst MR (2022). Transcriptome analysis of blastoderms exposed to prolonged egg storage and short periods of incubation during egg storage. BMC Genomics, 23(1): 260-261. DOI: https://www.doi.org/10.1186/s12864-022-08463-2

Campbell TW (1995). Avian haematology and cytology, 2nd Edition. Iowa State University Press., Ames, pp. 104-105. Available at: https://archive.org/details/avianhematologyc02edcamp

Chen J, Thesmar H, and Kerr WL (2005). Outgrowth of salmonellae and the physical property of albumen and vitelline membrane as influenced by egg storage conditions. Journal of Food Protection, 68(12): 2553-2558. DOI: https://www.doi.org/10.4315/0362-028x-68.12.2553

Chung HS and Lee KW (2014). Effect of Hen Age, Storage Duration and Temperature on Egg Quality in Laying Hens. International Journal of Poultry Science, 13(11): 634-636. Available at: https://www.cabidigitallibrary.org/doi/full/10.5555/20153087645

Damaziak K, Pawęska M, Gozdowski D, and Niemiec J (2018). Short periods of incubation, egg turning during storage and broiler breeder hens age for early development of embryos, hatching results, chick quality and juvenile growth. Journal of Poultry Science, 97(9): 3264-3276. DOI: https://www.doi.org/10.3382/ps/pey163

Debonne M, Baarendse P, Brand HVD, Kemp B, Bruggeman V, and Decuypere E (2008). Involvement of the hypothalamic-pituitary-thyroid axis and its interaction with the hypothalamic-pituitary-adrenal axis in the ontogeny of avian thermoregulation: A review. Journal of World's Poultry Science, 64(3): 309-321. DOI: https://www.doi.org/10.1017/s0043933908000056

Decuypere E, Tona K, Bruggeman V, and Bamelis F (2001). The day-old chick: A crucial hinge between breeders and broilers. Journal of World's Poultry Science, 57: 127-138. DOI: https://www.doi.org/10.1079/wps20010010

Doumas BT, Watson WA, and Biggs HG (1971). Albumin standards and the measurement of serum albumin with bromocresol green. Clinica Chimica Acta, 31: 87-96. DOI: https://www.doi.org/10.1016/0009-8981(71)90365-2

Dunnington EA and Siegel PB (1984). Thermoregulation in newly hatched chicks. Journal of Poultry Science, 63(7): 1303-1313. DOI: https://www.doi.org/10.3382/ps.0631303

Ebeid TA, Twfeek FA, Assar MH, Bealish A, El-Karim REA, and Ragab M (2017). Influence of pre-storage incubation on hatchability traits, thyroid hormones, antioxidative status and immunity of newly hatched chicks at two chicken breeder flock ages. Animal, 11(11): 1966-1974. DOI: https://www.doi.org/10.1017/s1751731117000738

El-Garhy OH (2021). Effect of short period of incubation during egg storage (SPIDES), storage period and flock age on productivity of post-hatching broiler chickens. Annals of Agricultural Science, Moshtohor, 59(3): 743-754. DOI: https://www.doi.org/10.21608/assjm.2021.207306

Elmenawey MA (2019). Effect of heat treatments during hatching eggs storage on hatchability traits and chick quality. Egyptian Poultry Science Journal, 39(4): 791-808. DOI: https://www.doi.org/10.21608/epsj.2019.63534

El-Prollosy A, Iraqi E, Elsayed N, Khalil H, El-Saadany A, and El-Sabrout K (2024). Impact of thermal manipulation during embryogenesis on thermotolerance and semen quality of mandarah roosters exposed to heat stress. Veterinary World, 17(6): 1311-1317. DOI: https://www.doi.org/10.14202/vetworld.2024.1311-1317

Fasenko GM, Robinson FE, Whelan AI, Kremeniuk KM, and Walker JA (2001). Prestorage incubation of long-term stored broiler breeder eggs: 1. Effects on hatchability. Journal of Poultry Science, 80(10): 1406-1411. DOI: https://www.doi.org/10.1093/ps/80.10.1406

Favresse J, Burlacu MC, Maiter D, and Gruson D (2018). Interferences with thyroid function immunoassays: clinical implications and detection algorithm. Endocrine Reviews, 39(5): 830-850. DOI: https://www.doi.org/10.1210/er.2018-00119

Fernandes EAN, Raymundo A, Martins LL, Lordelo M, and Almeida AM (2023). The naked neck gene in the domestic chicken: a genetic strategy to mitigate the impact of heat stress in poultry production—A review. Animals, 13(6): 1007. DOI: https://www.doi.org/10.3390/ani13061007

Gan JK, Jiang LY, Kong LN, Zhang XQ, and Luo QB (2015). Analysis of genetic diversity of the heat shock protein 70 gene on the basis of abundant sequence polymorphisms in chicken breeds. Genetics and Molecular Research, 14(1): 1538-1545. DOI: https://www.doi.org/10.4238/2015.march.6.1

Gharib H (2013). Effect of pre-storage heating of broiler breeder eggs, stored for long periods, on hatchability and chick quality. Egyptian Journal of Animal Production, 50(3): 174-184. Available at: https://www.ejap.journals.ekb.eg/article.pdf

Guo Y, Lillie M, Zan Y, Beranger J, Honaker CF, Siegel PB, Martin A, and Carlborg Ö (2019). A genomic inference of the white plymouth rock genealogy. Journal of Poultry Science, 98(11): 5272-5280. DOI: https://www.doi.org/10.3382/ps/pez411

Hamidu JA, Rieger AM, Fasenko GM, and Barreda DR (2010). Dissociation of chicken blastoderm for examination of apoptosis

and necrosis by flow cytometry. Journal of Poultry Science, 89(5): 901-909. DOI: https://www.doi.org/10.3382/ps.2009-00552

Haugh RR (1937). The haugh unit for measuring egg quality. United States Egg Poultry Magazine, 43(1): 552-573. Available at: https://www.sid.ir/paper/543065/fa.

Hemida MA, Abdel-Fattah SA, Madkour M, Aboelenin MM, Ahmed SY, and Shourrap M (2023). Hepatic heat shock proteins, antioxidant-related genes, and immunocompetence of heat-stressed broilers in response to short periods of incubation during egg storage and thermal conditioning. Journal of Thermal Biology, 116: 103640. DOI: https://www.doi.org/10.1016/j.jtherbio.2023.103640

Jiang B, Liang P, Deng G, Tu Z, Liu M, and Xiao X (2011). Increased stability of Bcl-2 in HSP70-mediated protection against apoptosis induced by oxidative stress. Cell Stress and Chaperones, 16(2): 143-152. DOI: https://www.doi.org/10.1007/s12192-010-0226-6

Jin Y, Lee KT, Lee WI, and Han YK (2010). Effects of storage temperature and time on the quality of eggs from laying hens at peak production. Asian-Australasian Journal of Animal Sciences, 24(2): 279-284. DOI: https://www.doi.org/10.5713/ajas.2011.10210

Joshua BI, Luka HS, Elisha IL, Nyam LS, Suleiman I, Gambo RA, and Samuel NS (2022). Haematological and serum biochemical parameters of broilers slaughtered at Bukuru live bird market of JOS south local government plateau state Nigeria. Acta Scientific Veterinary Sciences, 4(6): 5-11. DOI: https://www.doi.org/10.31080/asvs.2022.04.0399

Khan MJ, Khan SH, Bukhsh A, Abbass MI, and Javed M (2013). Effect of different storage period on egg weight, internal egg quality and hatchability characteristics of Fayumi eggs. Italian Journal of Animal Science, 12(2): e51. DOI: https://www.doi.org/10.4081/ijas.2013.e51

Khosravinia H (2015). Effects of intra-yolk sac inoculation of olive oil on physiological adaptive responses in newly hatched broiler chicks subjected to neonatal fasting. The Journal of Poultry Science, 52(4): 304-311. DOI: https://www.doi.org/10.2141/jpsa.0140120

Kong F, Wang H, Guo J, Peng M, Ji H, Yang H, Liu B, Wang J, Zhang X, and Li S (2016). Hsp70 suppresses apoptosis of BRL cells by regulating the expression of Bcl-2, cytochrome C, and caspase 8/3. In Vitro Cellular & Developmental Biology-Animal, 52: 568-575. DOI: https://www.doi.org/10.1007/s11626-016-0005-5

Küçükyılmaz K, Bozkurt M, Herken EN, Çınar M, Çatlı AU, Bintaş E, and Çöven F (2012). Effects of rearing systems on performance, egg characteristics and immune response in two-layer hen genotype. Asian-Australasian Journal of Animal Sciences, 25(4): 559-568. DOI: https://www.doi.org/10.5713/ajas.2011.11382

Mahmud A, Khan MZU, and Javed MA (2011). Effect of different storage periods and temperatures on the hatchability of broiler breeder eggs. Pakistan Veterinary Journal, 31(1): 78-80. Available at: http://www.pvj.com.pk/pdf-files/31_1/78-80.pdf

Maman AH and Yildirim I (2022). The effect of short periods of incubation during egg storage (SPIDES) on internal egg quality, hatchability and chick quality of long stored old layer breeder eggs. European Poultry Science, 86(353): 1-12. DOI: https://www.doi.org/10.1399/eps.2022.353

Maman AH, Özlü S, Uçar A, and Elibol O (2019). Effect of chick body temperature during post-hatch handling on broiler live performance. Journal of Poultry Science, 98(1): 244-250. DOI: https://www.doi.org/10.3382/ps/pey395

Maxine BM (1961). Outline of veterinary clinical pathology, 2nd Edition, pp. 350-361. Available at: https://www.cabidigitallibrary.org/action

Merck veterinary manual (2012). Haematologic reference ranges, pp. 234-240. Available at: https://www.merckvetmanual.com/hematology-reference-ranges

Nicholson D, French N, Tullett S, van Lierde E, and Jun G (2013). Short periods of incubation during egg storage– SPIDES. Lohmann Information, 48(2): 51-61. Available at: https://www.citeseerx.ist.psu.edu/documen

Nilsson J and Persson I (2004). Postnatal effects of incubation length in mallard and pheasant chicks. Oikos, 105(3): 588-594. DOI: https://www.doi.org/10.1111/j.0030-1299.2004.12594

Oguntoye MA, Idowu OMO, Sogunle OM, Akintunde AR, and Danladi Y (2018). Haematology and serum chemistry of finisher broiler chickens fed maize-cassava diet supplemented with methionine and inorganic sulphur. Nigerian Journal of Animal Science, 20(1): 152-162. Available at: https://www.ajol.info/index.php/tjas/article/view/166094

Okasha HM, El-Gendi GM, and Eid KM (2023). The effect of storage periods and SPIDES on embryonic mortality, hatching characteristics, and quality of newly hatched chicks in broiler eggs. Tropical Animal Health and Production, 55(2): 133. DOI: https://www.doi.org/10.1007/s11250-023-03547-x

Ouchi Y, Chowdhury VS, Cockrem JF, and Bungo T (2022). Thermal conditioning can improve thermoregulation of young chicks during exposure to low temperatures. Frontiers in Animal Science, 3: 919416. DOI: https://www.doi.org/10.3389/fanim.2022.919416

Özlü SERDAR, Elibol OKAN, and Brake J (2018). Effect of storage temperature fluctuation on embryonic development and mortality, and hatchability of broiler hatching eggs. Journal of Poultry Science, 97(11): 3878-3883. DOI: https://www.doi.org/10.3382/ps/pey253

Özlü S, Uçar A, Erkuş T, Yasun S, Nicholson AD, and Elibol OKAN (2021). Effects of flock age, storage temperature, and short period of incubation during egg storage on the albumen quality, embryonic development and hatchability of long-stored eggs. British Poultry Science, 62(4): 611-619. DOI: https://www.doi.org/10.1080/00071668.2021.1887454

Page JL, Nord A, Dominoni DM, and McCafferty DJ (2022). Experimental warming during incubation improves cold tolerance of blue tit (*Cyanistes caeruleus*) chicks. Journal of Experimental Biology, 225(10): jeb243933. DOI: https://www.doi.org/10.1242/jeb.243933

Reijrink I, Berghmans D, Meíjerhof R, Kemp B, and Brand HV (2010). Influence of egg storage time and preincubation warming profile on embryonic development, hatchability, and chick quality. Journal of Poultry Science, 89(6): 1225-1238. DOI: https://www.doi.org/10.3382/ps.2009-0018

Ritchie BW, Harrison JG, and Harrison RL (1994). Avian medicine: Principles and application. Lake Worth, Wingers Publishing, Florida, pp. 1840-9606. Available at: https://vetbooks.ir/avian-medicine-principles-and-application/

Rocha JSR, Baião NC, Barbosa VM, Pompeu MA, Fernandes MNS, Lara LJC, Matias CFQ, and Batista JVMSP (2013). Negative effects of fertile egg storage on the egg and the embryo and suggested hatchery management to minimise such problems. World's Poultry Science Journal, 69(1): 35-44. DOI: https://www.doi.org/10.1017/S0043933913000044

Romão JM, Moraes TGV, Teixeira RSC, Cardoso WM, and Buxade CC (2008). Effect of egg storage length on hatchability and weight loss in incubation of egg and meat type Japanese quails. Brazilian Journal of Poultry Science, 10: 143-147. https://www.doi.org/10.1590/S1516-635X2008000300001

Romijn C (1954). Development of heat regulation in the chick. Proceedings of Xth World's Poultry Congregation. Edinburgh Publishing Press, pp. 181-184. Available at: https://www.cabidigitallibrary.org/doi/full/10.5555/19552201495

Roschlau PV, Bernt E, and Gruber W (1974). Enzymatische bestimmung des gesamt-cholesterins im serum. Clinical Chemistry and Laboratory Medicine, 12(9): 403-407. DOI: https://www.doi.org/10.1515/cclm.1974.12.9.403

Ruiz J and Lunam CA (2002). Effect of pre-incubation storage conditions on hatchability, chick weight at hatch and hatching time in broiler breeders. British Poultry Science, 43(3): 374-383. DOI: https://www.doi.org/10.1080/00071660120103648

Scott TA and Silversides FG (2001). Effect of storage and layer age on quality of eggs from two lines of hens. Journal of Poultry Science, 80: 1240-124. DOI: http://www.doi.org/10.1093/ps/80.8.124

Sekeroglu A, Sarica M, Demir E, Ulutas Z, Tilki M, and Saatci M (2008). The effects of housing system and storage length on the quality of eggs produced by two lines of laying hens. Archiv Fur Geflugelkunde, 72: 106-109. Available at: https://www.acikerisim.balikesir.edu.tr/xmlui/handle/20.500.12462/8508

Simaraks S, Chinrasri O, and Aengwanich W (2004). Haematological, electrolyte and serum biochemical values of the Thai indigenous chickens (*Gallus domesticus*) in Northeastern, Thailand. Songklanakarin Journal of Science and Technology, 26(3): 425-430. Available at: https://www.citeseerx.ist.psu.edu/document

Suresh PK, Raj K, Thadathil N, Pal GK, and Sakhare PZ (2015). Application of chitosan for improvement of quality and shelf life of table eggs under tropical room conditions. Journal of Food Science and Technology, 52(10): 6345-6354. DOI: https://www.doi.org/10.1007/s13197-015-1721-7

Tanizawa H, Shiraishi J, Kawakami S, Tsudzuki M, and Bungo T (2014). Effect of short-term thermal conditioning on physiological and behavioural responses to subsequent acute heat exposure in chicks. The Journal of Poultry Science, 51(1): 80-86. DOI: https://www.doi.org/10.2141/jpsa.0130040

Tona K, Bamelis F, De Ketelaere B, Bruggeman V, Moraes VMB, Buyse J, and Decuypere E (2003). Effects of egg storage time on the spread of hatch, chick quality, and chick juvenile growth. Journal of Poultry Science, 82(5): 736-741. DOI: https://www.doi.org/10.1093/ps/82.5.736

Trinder P (1969). Determination of blood glucose using an oxidase-peroxidase system with a noncarcinogenic chromogen. Journal of Clinical Pathology, 22(2): 158-161. DOI: https://www.doi.org/10.1136/jcp.22.2.158

Underwood G, Andrews D, Phung T, and Edwards LE (2021). Incubation, hatchery practice and the welfare of layer hens. Animal Production Science, 61(10): 867-875. DOI: https://www.doi.org/10.1071/an20391

Varguez-Montero G, Sarmiento-Franco L, Santos-Ricalde R, and Segura-Correa J (2012). Egg production and quality under three housing systems in the tropics. Tropical Animal Health and Production, 44(2): 201-204. DOI: https://www.doi.org/10.1007/s11250-011-0006-z

Varley H, Owen M, and Bell C (1980). Practical clinical biochemistry. William and Helnemann Medical Publishing Press., London, UK, pp. 551-552. Available at: https://www.cabidigitallibrary.org/doi/full/10.5555/19621404878

Wang Q, Jin G, Wang N, Guo X, Yongguo J, and Ma M (2017). Lipolysis and oxidation of lipids during egg storage at different temperatures. Czech Journal of Food Sciences, 35(3): 229-235. DOI: https://www. doi.org/10.17221/174/2016-cjfs

Wekstein DR and Zolman JF (1969). Ontogeny of heat production in chicks. Federation Proceedings, 28(3): 1023-1028. Available at: https://europepmc.org/article/med/5783499

Willemsen H, Li Y, Willems E, Franssens L, Wang Y, Decuypere E, and Everaert N (2011). Intermittent thermal manipulations of broiler embryos during late incubation and their immediate effect on the embryonic development and hatching process. Journal of Poultry Science, 90(6): 1302-1312. DOI: https://www.doi.org/10.3382/ps.2011-01390

Xin H and Rieger SR (1995). Physical conditions and mortalities associated with international air transport of young chicks. Transactions of American Society of Agricultural and Biochemical Engineers, 38(6): 1863-1867. DOI: https://www.doi.org/10.13031/2013.28015

Yahav S and Hurwitz S (1996). Induction of thermotolerance in male broiler chickens by temperature conditioning at an early age. Journal of Poultry Science, 75: 402-406. DOI: https://www.doi.org/10.3382/ps.0750402

Yahav S and McMurtry J (2001). Thermotolerance acquisition in broiler chickens by temperature conditioning early in life, the effect of timing and ambient temperature. Journal of Poultry Science, 80: 1662-1666. DOI: https://www.doi.org/10.1093/ps/80.12.1662

Yahav S, Rath RS, and Shinder D (2004). The effect of thermal manipulations during embryogenesis of broiler chicks (*Gallus domesticus*) on hatchability, body weight and thermoregulation after hatch. Journal of Thermal Biology, 29(4-5): 245-250. DOI: https://www.doi.org/10.1016/j.jtherbio.2004.03.002

Yamak US, Sarıca M, Erensoy K, and Ayhan V (2020). The effects of storage conditions on quality changes of table eggs. Journal of Consumer Protection and Food Safety, 16(1): 71-81. DOI: https://www.doi.org/10.1007/s00003-020-01299-6

Yeboah PP, Konadu LA, Hamidu JA, Poku EA, Wakpal D, Kudaya PY, and Siddiq SM (2019). Comparative analysis of hatcheries' contribution to poor development of day-old chicks based on biological and immunological performance. Veterinary World, 12(11): 1849. DOI: https://www.doi.org/10.14202/vetworld.2019.1849

Zita L, Tůmová E, and Štolc L (2009). Effects of genotype, age and their interaction on egg quality in brown-egg laying hens. Acta Veterinaria Brno, 78(1): 85-91. DOI: https://www.doi.org/10.2754/avb200978010085