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Effects of short heating periods during egg storage on quail embryonic development, incubation performance, chick quality, and chick performance up to 35 days

Efeitos de curtos períodos de aquecimento durante o armazenamento de ovos no desenvolvimento embrionário de codornas, desempenho de incubação, qualidade do pintinho e desempenho do pintinho até 35 dias de idade

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Highlights

Fertile Japanese quail eggs were subjected to pre-heating during the storage period.
Three storage periods and two pre-heating periods were tested.
Treatments did not affect egg quality, incubation or progeny performance.

Abstract

This study investigated the effects of pre-heating fertile Japanese quail eggs during storage on embryonic development, incubation performance, hatched chick quality, and chick performance up to 35 days of age. The experiment was laid out in a completely randomized design in a 3 × 2 + 1 factorial arrangement, totaling seven treatments. These treatments included three storage periods (3.5, 6.5, and 9.5 days) and two pre-heating periods (0 and 4 h at 37.5°C and 60% RH). Pre-heating was conducted on the third day of egg storage inside the incubator machine, with the control treatment involving the storage of fertile eggs for 12 h without pre-heating. Extended egg storage for more than six days led to an increase in the percentages of yolk and shell, elevated albumen pH, reduced albumen percentage, diminished hatchability rate, and an increased embryonic mortality rate. Pre-heating the eggs resulted in a reduction in the weight, length, and amount of residual yolk sac of the newly hatched chick. No significant effects of pre-heating fertile Japanese quail eggs during storage were observed on the studied variables. Therefore,

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the protocol involving a 4-h pre-heating at 37.5 ºC and 60% relative humidity during the storage period does not yield improvements in incubation rates or chick quality in Japanese quail. Further studies are warranted to determine the optimal protocol for pre-heating Japanese quail eggs.
Key words: Embryo. Hatch. Pre-heating. Progeny.

Resumo

Os efeitos do pré-aquecimento de ovos férteis de codorna japonesa durante o armazenamento foram analisados no desenvolvimento embrionário, desempenho de incubação, qualidade do pintinho eclodido e desempenho do pintinho até 35 dias de idade. O experimento foi conduzido em delineamento inteiramente casualizado em esquema fatorial 3 × 2 + 1, totalizando sete tratamentos, com três períodos de armazenamento de 3,5, 6,5 e 9,5 dias e dois períodos de pré-aquecimento de 0 e 4 horas a 37,5°C e 60% UR. O pré-aquecimento foi realizado no terceiro dia de armazenamento dos ovos dentro da incubadora. O tratamento controle foi o armazenamento dos ovos férteis por 12 horas sem pré-aquecimento. O armazenamento dos ovos por um período superior a seis dias causou aumento na porcentagem de gema e casca, aumento no pH do albúmen, redução na porcentagem de albúmen, redução na taxa de eclodibilidade e aumento na taxa de mortalidade embrionária. O aquecimento dos ovos ocasionou redução no peso, comprimento e quantidade de gema residual no pintinho recém-eclodido. Não houve efeito do pré-aquecimento dos ovos férteis de codornas japonesas durante o armazenamento sobre as variáveis estudadas. Portanto, o protocolo de pré-aquecimento por 4 horas a 37,5ºC e 60% de umidade relativa durante o período de armazenamento não produz melhorias nas taxas de incubação ou na qualidade do pintinho em codornas japonesas, sendo necessários mais estudos para determinar o melhor protocolo de pré-aquecimento de ovos de codornas japonesas.
Palavras-chave: Eclosão. Embrião. Pré-aquecimento. Progênie.

Introduction

Incubation is a critical stage in the poultry production system, and its efficiency is dependent on various environmental factors. Notably, the storage period of fertile eggs plays a major role, especially as the industry increasingly faces challenges with the capacity of incubation unable to keep up with the growing number of chicks, leading to extended egg storage (Damaziak et al., 2021). Prolonged storage can compromise chick quality and hatchability rates (Ayeni et al., 2020). Scientific evidence indicates that extended storage induces cell death by both necrosis and apoptosis of embryonic cells, causing a delay in embryonic growth recovery. This phenomenon can occur even under ideal incubation conditions, resulting in a slow embryonic growth rate (Fasenko, 2007), with more than half of the cells present during egg laying dying after 10-12 days of storage (Bakst et al., 2012). During this period, changes in egg characteristics, such as an increase in albumen pH (Gharib, 2013) and a reduction in Haugh unit and egg weight (Uyanga et al., 2020), contribute to negative effects on avian embryo viability.

In response to these challenges, the egg heat treatment technique has been developed. This approach involves applying short incubation periods during the storage period to minimize the adverse

effects of prolonged storage on incubation performance. The application of this technique enables the embryo to reach the hypoblast stage, considered a phase in early embryonic development when the embryo becomes more resistant to the storage period (Reijrink et al., 2009). A more resistant embryo can lead to improved hatchability rates, enhanced quality of incubated birds, and a reduction in total and early embryonic mortality rates for eggs from broiler breeders (Abdel-Halim et al., 2015; Gucbilmez et al., 2013). However, the application of this technique before and during the storage of fertile Japanese quail eggs does not yield similarly comprehensive results and requires further studies, as it has been shown to reduce chick weight and narrow the hatching window (Damaziak et al., 2021).

In light of these considerations, this study aims to investigate the influence of the technique of pre-heating fertile eggs during the storage period on egg quality, embryonic development of Japanese quail, incubation performance, the quality of hatched chicks, and chick performance up to 35 days of age.

Materials and Methods

The research received approval from the Ethics Committee on the Use of Animals in Experimentation at the State University of Maringa/Maringa/PR (approval no. 4117071220).

Animals and management

The experiment was conducted at the Iguatemi Experimental Farm (FEI) of the State University of Maringá (UEM), in the state of Parana, Brazil (23°21' S, 52°04' W, altitude of 564 m), during the months of February, March, and April, covering the seasons of summer and fall.

Breeders, selected based on weight and laying rate, comprised a total of 800 Japanese quail (600 females and 200 males) at 15 weeks of age. They were housed in metal galvanized iron cages (25 × 39 cm) with nipple drinkers and trough feeders at a ratio of six females to two males. Throughout the experimental period, feed and water were provided *ad libitum*, with a 17-h light program. The diet, formulated based on corn and soybean meal, adhered to the composition of ingredients and nutritional requirements of quail during the laying phase according to Brazilian tables (Rostagno et al., 2017). Daily temperature and humidity measurements inside the laying house recorded mean maximum temperatures of 34.43 ± 1.82 ºC, minimum temperatures of 24.40 ± 4.11 ºC, maximum humidity of 65.81 ± 7.44%, and minimum humidity of 50.71 ± 7.70%, using a digital thermo-hygrometer.

Egg collection and storage

Eggs were collected from breeders immediately after laying (14h00 to 16h00), at three-day intervals. For each storage period (control, at 12 h; and 3.5, 6.5, and 9.5 days), 200 eggs were selected based on weight, size, and quality (very small, broken, or cracked eggs and soft shells were discarded) and then placed in incubator trays with a round base, stored in a cooled room with air conditioning. The room temperature, monitored daily with a digital thermometer, recorded mean

maximum temperatures of 20.59 ± 0.51 ºC, minimum temperatures of 19.43 ± 0.59 ºC, maximum humidity of 69.48 ± 5.53%, and minimum humidity of 53.51 ± 5.67%.

Experimental design and egg pre-heating

The experimental design was entirely randomized in a 3 × 2 + 1 factorial arrangement (days of storage × hours of pre-heating), totaling seven treatments with 200 eggs per treatment. Additionally, a control treatment involved 12 h of storage with no pre-heating. Egg storage durations were 3.5, 6.5, and 9.5 days. These eggs were stored in a room at a mean temperature of 20 °C. On the third day of storage for each period, the eggs underwent pre-heating for 0 or 4 h inside automatic incubators set at 37.5 °C air temperature and 60% relative humidity. Subsequently, the warmed eggs were cooled until reaching approximately 24 °C (around one hour) and then returned to the egg storage room at 20 °C until the incubation process commenced.

Analysis of egg quality and albumen pH

Egg quality was assessed in 20 randomly selected eggs per treatment on the day of incubation. The following variables were analyzed: egg weight; shell, albumen, and yolk percentages (%); albumen and yolk heights (mm); Haugh unit (HU); yolk index (YI); and specific gravity (g ml^{-1}). The eggs were individually identified, weighed on precision electronic scales, and their specific gravity (g ml^{-1}) was determined. Albumen (mm) and yolk (mm) heights were measured using a digital pachymeter (Digimess, accurate to 0.02 mm) to calculate HU and YI. Haugh unit was determined according to the equation proposed by Haugh (1937): $HU = 100 \log (H + 7.57 - 1.7 W^{0.37})$, where HU = Haugh unit, H is the height of the albumen (mm), and W represents the weight of the egg (g). The yolk index was determined as the ratio between yolk height (mm) and yolk diameter (mm).

The pH of egg albumen was analyzed in 20 eggs per treatment on the day of incubation. The pH meter probe was standardized using a pH 7.00 buffer solution, and the pH of the egg albumen was measured using the digital pH meter probe (Simpla, model pH 140). Between measurements, the probe was cleaned with distilled water.

Analysis of embryonic development

To analyze the stage of embryonic development after storage periods (12 h; 3.5, 6.5, and 9.5 days), 20 eggs were collected from each treatment. These were washed in a phosphate buffer solution and fixed in 2.5% glutaraldehyde, pH 7.4, 0.1M PBS solution (Gupta & Bakst, 1993). After washing, fixative was applied over the embryo, and moments later, the embryo was isolated and placed into the same fixative solution for storage.

After collection, the embryos were analyzed under stereomicroscopy with a digital camera and coupled digital analysis software (Motic®) in the laboratory. Developmental stages were identified using the scale described by Eyal-Giladi and Kochav (EGK) (1976), with Roman numerals from I to XIV, converted to corresponding Arabic numbers for statistical analysis.

Analyses of incubation performance, progeny quality, and organ morphology

Following the storage period (12 h; 3.5, 6.5, and 9.5 days), the eggs were incubated in an automatic incubator (Petersime®, model Labo 13) adjusted to 60% humidity and 37.4 ºC temperature, with automatic turning every 60 min (capacity 3,978 quail eggs). After 348 h of incubation, the eggs were transferred to the hatcher (Petersime®, model Labo 9) for another 72 h, adjusted to 37.0 °C and 70% humidity. Post-incubation, hatched and unhatched eggs were counted, and the following incubation variables were calculated: total hatchability rate, early mortality rate, intermediate mortality rate, and late mortality rate. Unhatched eggs were opened to determine the mortality rate, classified into total mortality and by period (initial + average: 0-11 d and late: 12 d until hatching + pecked-shell eggs unhatched). The following equations were used to obtain these indices:

Total hatchability rate (%) = (Hatched chicks (n) / Incubated eggs (n)) × 100;

Total mortality rate (%) = (Total unhatched chicks (n) / Fertile eggs (n)) × 100;

Initial mortality rate (%) = (Total early unhatched chicks (n) / Fertile eggs (n)) × 100; and

Late mortality rate (%) = (Total late unhatched chicks (n) / Fertile eggs (n)) × 100.

Chick quality was assessed in 50 one-day-old chicks per treatment. Chicks were categorized using the Pasgar® Score, a scale ranging from 1 to 10, evaluating reflexes, umbilical scar quality, beak, abdomen, umbilicus, and legs. Points were deducted for each identified irregularity (Boerjan, 2002). The weight (g) and length (measured from the middle phalanx to the beak) of the newly hatched chick were recorded using a metric ruler.

Offal morphology was examined in 20 one-day-old chicks per treatment. Birds were anesthetized with isoflurane (3%), and once unconsciousness was confirmed by the loss of reflexes, they were euthanized by cervical dislocation. Morphological data were collected to determine developmental traits, including the weights of residual yolk sac (g) and offal (heart, proventriculus, gizzard, intestine, and liver) (mg).

Quail productive performance

Eggs were incubated under the aforementioned conditions, and, post-hatching, unsexed chicks were selected and distributed among treatments in an entirely randomized design, following a 2 × 3 + control factorial arrangement with four replicates (boxes) of 20 birds each. Performance was assessed based on daily weight gain (g), feed intake (g), and feed conversion during the periods of 1 to 35 days of age, 1 to 14 days of age (starter phase), and 15 to 35 days of age (grower phase).

Chicks were housed in 2.5 × 1 m boxes with rice husk bedding. Chick drinkers were used initially, and later, water was supplied through bell drinkers. Infrared lamps maintained an initial temperature of 35 °C for the first 15 days, gradually reduced to room temperature. Feed was provided *ad libitum* in tube feeders, and the birds were kept under

natural lighting. The diet was consistent across treatments, focusing on evaluating only the residual effects of storage and pre-heating applied to the eggs. Diets in the starter and grower phases followed nutritional requirements for quail defined in the Brazilian Tables for Poultry and Swine (Rostagno et al., 2017).

Statistical analysis

Egg quality, chick quality, and quail performance data underwent analysis of variance (ANOVA) using the general linear model (GLM) procedure of Statistical Analysis System [SAS] (2008) at a 5% significance level to describe the influence of pre-heating and days of egg storage. The means of treatments with and without pre-heating were compared to the mean of control treatment using Dunnett's test, also at a 5% significance level within the SAS program.

The likelihood of fertility, hatchability, total mortality, and initial or late mortality was analyzed using GENMOD procedures in SAS, employing a binomial distribution and LOGIT link function. The means for total hatchability, fertile eggs, and total mortality in the treatments involving storage with and without heating were compared to the control treatment through contrast analysis at a 5% significance level in SAS.

Results and Discussion

None of the parameters related to egg quality, embryonic development, incubation performance, organ morphometry, or chick quality and productivity were significantly affected ($p>0.05$) by the interaction between pre-heating and days of storage.

Egg quality

Egg weight decreased with increasing days of storage and pre-heating. Pre-heating led to a reduction in Haugh unit and yolk index, whereas storage days reduced albumen (%) and increased yolk (%) and shell (%) ($p<0.05$). Additionally, albumen pH increased with storage days and pre-heating ($p<0.05$) (Table 1).

Comparing the results of internal and external egg quality after 3.5, 6.5, and 9.5 days of storage with or without pre-heating to the control group (12 h storage without pre-heating), through Dunnett's test, we observed that only eggs stored for 3.5 days did not show significantly inferior results in egg weight ($p<0.05$). This finding aligns with Ondrušíková et al. (2018), who noted a gradual reduction in the weight of quail eggs from 12.27 to 11.67 g during an eight-day storage period. Nowaczewski et al. (2010) also reported a reduction in quail egg weight after storage (3, 5, and 8 days) at a temperature of 19 °C and 50-55% relative humidity compared to freshly laid eggs. Abdel-Halim et al. (2015) found a significantly higher egg weight loss percentage in eggs pre-heated for six hours and stored for 21 days.

In addition to weight loss, there was a decrease in the specific gravity of Japanese quail eggs subjected to storage and pre-heating. Nowaczewski et al. (2010) detected lower specific gravity in Japanese quail eggs stored for five to eight days in a refrigerated environment compared to freshly laid eggs,

describing a reduction in gravity of 1.065 to 1.039 g cm-³. This reduction is attributed to water loss through evaporation after laying, which leads to a progressive increase in the air chamber and, consequently, a reduction in egg specific gravity (M. S. V. Santos et al., 2009; Freitas et al., 2011).

Regarding gravity and yolk index, eggs stored for 3.5, 6.5, and 9.5 days with or without pre-heating showed inferior results compared to the control group ($p<0.05$). As for Haugh unit, only eggs subjected to 9.5- and 6.5-day storage with pre-heating exhibited lower values than the control group ($p<0.05$). This is consistent with the findings of Northcutt et al. (2022), who observed a linear decrease in Haugh unit values with increased storage time of quail eggs (85.30 to 76.30) immediately after laying for 120 days. The reduction in Haugh unit is attributed to a decrease in the height of the albumen, resulting from the breakdown of the ovomucin-lysozyme complex during storage, which leads to an increase in egg pH (Akter et al., 2014).

The albumen (%) of the eggs subjected to 9.5 days of storage without pre-heating, as well as the eggs that underwent pre-heating and were stored for 3.5, 6.5, and 9.5 days, showed significantly lower results than the control group ($p<0.05$). As for the shell (%) and yolk (%) variables, only the eggs subjected to pre-heating and stored for 3.5, 6.5, and 9.5 days exhibited significantly higher results than the control. Significantly lower yolk indices were also evident in eggs subjected to pre-heating. This reduction is attributed to the pre-heating temperature during the storage period. According to the European Food Safety Authority (EFSA) (2014), the temperature elevation during storage causes water transfer from the albumen to the yolk, promoting its enlargement and a reduction in height, leading to a decrease in the yolk index. Elmenawey (2019) observed a significant reduction in the yolk index of fertile eggs stored for five days and subjected to pre-heating.

In this study, albumen percentage declined with storage days. According to Lana et al. (2017), this phenomenon occurs in eggs subjected to prolonged storage periods, even under refrigeration conditions, as the enzymes present in the albumen hydrolyze the amino acid chains, destroying the protein structure and releasing water bound to large protein molecules, which causes the fluidization of the albumen. Abioja et al. (2021) found that eggs under four days of storage had a lower percentage of shell compared to eggs with 16 days of storage, which showed the highest percentage. This reduction in the percentage of eggshell is due to the decrease in the weight of albumen with the increase in storage period, making the shell represent a larger proportion of the egg.

In the present study, the pH of the albumen became more alkaline with storage, reaching 9.18 at 3.5 days of storage and 9.38 at the end of the storage period. Additionally, there was an increase from 9.24 to 9.35 in the pH of the albumen when the eggs were subjected to pre-heating. The increase in pH values of the albumen during storage is directly related to the loss of carbon dioxide to the external environment. H_2CO_3, one of the components of the buffer system of the albumen, dissociates, forming water (H_2O) and carbon dioxide (CO_2) (J. S. Santos et al., 2016). The CO_2 is released into the

environment, leading to an increase in the pH of the albumen. Northcutt et al. (2022) demonstrated in their study that there was an increase from 9.13 to 9.22 in the pH of stored quail eggs, even under refrigeration, during the 30-day period.

Özlü et al. (2018) reported that heating eggs for a short period during storage had no influence on egg albumen traits. However, eggs stored for 14 days at temperatures of 12, 15, and 18 °C showed increases in albumen pH values of 9.35, 9.39, and 9.42, respectively, as a function of storage days.

Embryonic development

There was no interaction effect between pre-heating and storage days on the stages of embryonic development, and no significant effect of storage days alone. However, pre-heating at 37.5 °C for 4 h accelerated the stage of development ($p<0.05$) (Table 1). On average, the embryos progressed from stage XI to XII. According to Özlü et al. (2018), the progression in embryonic development stage when eggs are pre-heated depends on the total exposure time of eggs to heating. The authors pre-heated fertile broiler eggs during storage, maintaining the eggshell temperature above 32 °C for 3.5 h, and found that pre-heated eggs reached a more advanced developmental stage (EGK 11.14) compared to the control group, which was not pre-heated (EGK 9.51).

Dymond et al. (2013) also suggested progression in embryo development after pre-heating fertile eggs during the storage period. The authors noted that embryos at oviposition were at stage EGK 10.5, and when subjected to 6 h of pre-heating at 37 °C, embryos from broiler breeders advanced to stage XIV (EGK 14), where hypoblast formation is complete. According to Reijrink et al. (2010), the beneficial effect of pre-heating during the storage of fertile eggs lies in the completion of embryo hypoblast formation, making the embryo less sensitive to changes in pH.

Among the embryos not pre-heated, 12 were in stage X, 62 in stage XI, and six in stage XII. Regarding those pre- heated for 4 h at 37.5°C, 11 were in stage XI, 41 in stage XII, and eight in stage XIII. Stage X embryos exhibited a completed formation of the zona pellucida, with a clearly demarcated region between the zona pellucida and the opaque area (Figure 1). The most posterior region of the zona pellucida remains as a transparent sickle-shaped band.

Table 1
Egg quality variables and embryonic development stages of Japanese quail eggs pre-heated before incubation and stored for different periods

Hours of pre-heating	Days of storage	Egg weight (g)	Specific gravity (g ml^{-1})	Haugh unit	Yolk index	Albumen (%)	Egg yolk (%)	Shell (%)	Albumen pH	EGK stage
Control		10,78	1074.25	89.25	0.50	62.27	30.01	7.75	7.70	11.00
0	3.5	10.34	1061.50*	88.07	0.45*	60.11	31.88	8.02	9.18*	11.00
	6.5	10.29*	1061.50*	87.48	0.43*	59.81	31.97	8.12	9.20*	11.12
	9.5	9.65*	1061.50*	86.92	0.42*	57.82*	33.28	8.18	9.28*	11.07
4	3.5	10.15*	1061.25*	86.74	0.42*	57.00*	34.13*	8.35*	9.35*	12.00
	6.5	10.00*	1061.00*	85.22*	0.41*	58.43*	34.32*	8.63*	9.38*	12.05
	9.5	9.41*	1060.50*	85.26*	0.41*	56.60*	34.49*	8.80*	9.41*	12.00
Mean		10.09	1063.04	87.00	0.44	58.91	32.83	8.27	9.07	11.46
SEM		0.059	0.452	0.365	0.005	0.405	0.377	0.062	0.050	0.060
0		10.09 a	1061.41	87.52 a	0.44 a	58.38	33.38	8.26	9.24 b	11.06 a
4		9.85 b	1060.91	85.74 b	0.41 b	58.28	33.25	8.47	9.35 a	12.00 b
	3.5	10.24 a	1061.37	86.65	0.43	59.97 a	31.92 b	8.10 b	9.23 b	11.50
	6.5	10.15 a	1061.12	87.10	0.42	58.14 ab	33.69 ab	8.24 b	9.29 ab	11.57
	9.5	9.53 b	1061.00	86.07	0.42	56.80 b	34.40 a	8.75 a	9.38 a	11.53
Source of variation				P-value						
Pre-heating		0.020	0.243	0.026	0.037	0.973	0.809	0.082	0.001	<0.0001
Storage		<0.0001	0.766	0.833	0.325	0.012	0.041	<0.0001	0.001	0.815
Interaction		0.923	0.710	0.567	0.526	0.869	0.851	0.953	0.310	0.906

EGK: stage of embryo development according to Eyal Giladi and Kochav (1976); SEM: standard error of the mean;
* Means of the stored treatments with and without heating were compared with the mean of the control treatment by Dunnett's test;
[a,b] Means within a row followed by different superscript letters are significantly different by Tukey's test (P < 0.05).

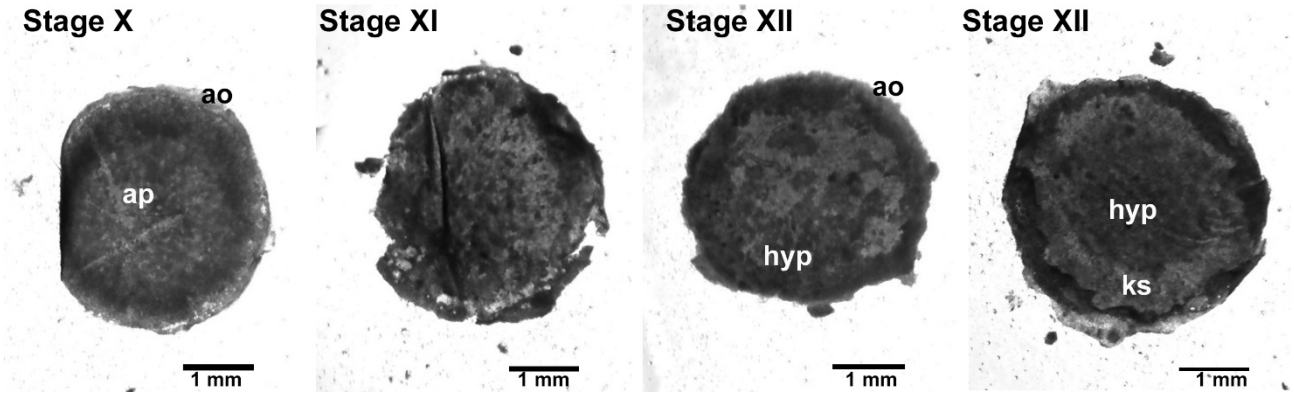

Figure 1. Japanese quail embryos classified into stages X, XI, XII, and XIII, as proposed by Eyal-Giladi and Kochav (1976). Area opaca (ao), area pellucida (ap), hypoblast (hyp), and Koller´s sickle (ks).

Embryos at stages XI and XII are classified as period C constituents according to Eyal-Giladi and Kochav (1976). At stage XI, embryos have a thin layer on the upper surface of the blastoderm through which deeper concentrations of cells can be seen. On the ventral side of the blastoderm, there is a posterior section of the opaque area resembling a transparent belt, where the anterior border is demarcated by a concentration of several individual and distinctly sized groups of cells. Together, they assume the horseshoe shape known as Koller's sickle, which comprises the beginning of the hypoblast. When stage XII is reached, the hypoblast is visualized covering half of the lower superficial layer of the zona pellucida, presenting a discontinuous form with the appearance of formation through the fusion of segmented cell masses. In stage XIII, the posterior margin of the hypoblast is very pronounced on the ventral side and can also be visualized on the dorsal side through the transparent epiblast, with a continuous upper surface (Figure 1).

Incubation performance, organ morphometry and chick quality and production performance

Incubation performance variables were not significantly influenced by the interaction between pre-heating and days of storage (Table 2). However, the days of egg storage led to a significant reduction in the probabilities of total and fertile hatching and an increase in total mortality ($p<0.05$). Fertility and infertility rates, early + medium mortality, and late mortality were not influenced by any of the factors under study. Contrast analysis revealed that fertile eggs stored for 3.5, 6.5, and 9.5 days, with or without pre-heating, displayed lower results for total and fertile hatching and total mortality rates when compared to the control group (12-h storage without pre-heating).

Table 2
Incubation performance of Japanese quail eggs pre-heated before incubation and stored for different periods

Hours of pre-heating	Days of storage	Set eggs, n	Fertility, %	Infertility, %	Hatchability, total eggs, %	Hatchability, fertile eggs, %	Mortality, %		
							Total	Early+Medium	Late+Hatch
Control		200	97.50	2.50	90.00	92.30	7.70	46.68	53.32
0		525	96.58	3.42	79.85	82.69	17.31	48.50	51.50
4		505	96.83	3.47	80.35	82.99	16.40	54.74	49.18
	3.5	375	97.33	2.67	83.48 a	85.78 a	14.22 a	40.69	59.31
	6.5	355	96.62	3.38	80.85 ab	83.67 ab	15.46 a	62.25	43.41
	9.5	300	96.05	3.95	75.34 b	78.47 b	21.53 b	51.77	48.23
Source of variation					P-value				12.00
Pre-heating			0.820	0.820	0.840	0.900	0.704	0.428	0.764
Storage			0.647	0.647	0.031	0.047	0.037	0.086	0.249
Interaction			0.858	0.858	0.789	0.838	0.974	0.791	0.380
					Estimated β [1]				
Control			3.663	-3.663	2.197	2.484	-2.484	-0.133	0.133
0			3.340	-3.340	1.377	1.564	-1.564	-0.060	0.060
4			3.420	-3.420	1.408	1.585	-1.629	0.190	-0.033
	3.5		3.596	-3.596	1.620	1.797	-1.797	-0.377	0.377
	6.5		3.352	-3.352	1.440	1.634	-1.699	0.500	-0.265
	9.5		3.192	-3.192	1.117	1.293	-1.293	0.071	-0.071
					Contrast analysis				
Control vs. 3.5 d					0.02	0.01	0.01		
Control vs. 6.5 d					0.003	0.003	0.007		
Control vs. 9.5 d					<0.0001	<0.0001	<0.0001		
Control vs. 0 h					0.0008	0.0007	0.0007		
Control vs. 4 h					0.001	0.001	0.001		

[1] Estimated β = were used to estimate the probabilities by the following formula: $y = 100 \times (\exp^{(\beta)}/1+\exp^{(\beta)})$.
[a-b] Different letters represent the differences between the storage or pre-heating that were detected (P <0.05) by the least-squares mean test.

According to Roriz et al. (2016), longer egg storage periods lead to a reduction in hatchability rates due to weight losses in the eggs during this period. Higher loss rates are observed when eggs are subjected to 10 days of storage, suggesting that greater weight loss may be related to worsened hatchability rates. In a study of Japanese quail breeders, Petek and Dikmen (2004) found lower total hatchability and fertility rates when eggs were stored for longer periods. The authors also noted that there was no influence of storage days or the interaction between storage days and pre-heating on early + medium and late + shell mortality rates. Aygun and Sert (2013), evaluating the hatchability of fertile eggs of Japanese quail, reported that eggs stored for 14 days had significantly lower hatchability rates (88.27%) than eggs stored for seven days, with the latter showing a rate of 91.54%. Hamza et al. (2020) concluded that storing broiler breeder eggs for a longer period of 14 days, compared to four days of storage, resulted in a significant linear decrease in hatchability of fertile and total eggs, indicating a higher percentage of total embryonic mortality.

In contrast to the present experiment, where the focus is on the incubation of Japanese quail eggs stored for long periods, Damaziak et al. (2021) employed a specific protocol to achieve favorable hatching results for eggs stored for extended periods, starting from 12 days. Their protocol involved pre-heating the eggs at a temperature of 37.8 °C and relative humidity between 50-55%, totaling 16 h over 12 days of storage. This pre-heating regimen included warming the eggs on the fifth day for two hours, on the seventh day for three hours, on the ninth day for five hours, and on the eleventh day for six hours. Concurrently, they implemented egg turning every 12 h throughout the entire period. Damaziak et al. (2021) noted that the application of pre-heating during the storage of eggs for short periods did not significantly affect hatchability rates. Additionally, the brief pre-heating technique at 37 °C for 6 h before storage has been identified as a method to potentially enhance total hatchability (Lotfi et al., 2011).

Variables related to progeny quality (chick weight, length, and Pasgar® score), residual yolk sac weight, and organ morphometry (heart, intestine, liver, proventricular, and gizzard weight) measured on the first day of chick life were not influenced by the interaction between pre-heating and days of storage (Table 3). However, pre-heating influenced a reduction in chick weight, length, and residual yolk sac weight ($p<0.05$). Progeny from eggs not subjected to pre-incubation heating during storage exhibited higher values for weight (7.09 g), length (11.18 mm), and residual yolk sac (0.37 g). Conversely, when eggs were subjected to pre-heating, there was a reduction in weight (6.82 g), length (10.67 mm), and residual yolk sac (0.28 g) values of the progeny.

When compared using Dunnett's test, the results of progeny quality, residual yolk sac weight, and morphometry of organs from newly hatched chicks from eggs stored for 3.5, 6.5, and 9.5 days, pre-heated or not, were contrasted with the results of the same variables in chicks from the control group (stored for 12 h without pre-heating). Chicks from eggs stored for 3.5, 6.5, and 9.5 days and subjected to the pre-heating process displayed significantly lower weights and lengths ($p<0.05$). Regarding residual yolk sac weight, chicks from eggs stored for 3.5, 6.5, and 9.5 days, pre-heated or not, showed significantly lower results than the control group ($p<0.05$).

Table 3
Chick quality (n=50) and organ morphometry (n=20) of Japanese quail chicks from eggs pre-heated before incubation and stored for different periods

		Chick quality			Organ morphometry					
Hours of pre-heating	Days of storage	Body weight (g)	Length (mm)	Pasgar® Score	Residual yolk sac (g)	Heart (mg)	Proventriculus (mg)	Intestine (mg)	Late+ Hatch	Liver (mg)
Control		7.33	11.39	9.68	0.46	99.50	119.60	431.50	389.85	195.05
0	3.5	7.15	11.22	9.62	0.38*	82.20	97.15	373.30*	346.40	176.70
	6.5	7.05	11.18	9.60	0.38*	79.55	95.30	347.65*	342.60	172.10
	9.5	7.05	11.15	9.62	0.34*	71.50	89.35	336.30*	324.95*	177.95
4	3.5	6.84*	10.71*	9.52	0.29*	68.50*	84.85	334.15*	316.35*	172.25
	6.5	6.85*	10.66*	9.46	0.28*	68.10*	78.15	333.30*	314.50*	178.25
	9.5	6.78*	10.66*	9.44	0.27*	67.25*	61.55	332.05*	313.30*	175.30
Mean		7.01	10.99	9.56	0.34	76.65	89.42		355.46	178.22
0		7.09 a	11.18 a	9.56	0.37 a	73.93	86.72	352.42	335.12	175.58
4		6.82 b	10.67 b	9.52	0.28 b	71.76	82.07	333.17	317.58	175.26
	3.5	7.00	10.97	9.62	0.34	69.94	80.21	356.51	338.59	173.97
	6.5	6.95	10.92	9.56	0.33	67.57	88.90	338.68	324.73	176.17
	9.5	6.91	10.91	9.45	0.31	80.75	83.98	333.76	316.29	176.07
SEM		0.035	0.240	0.033	0.011	0.002	0.006	0.006	0.005	0.002
Source of variation						*P*-value				
Pre-heating		0.0003	<0.0001	0.650	0.0001	0.724	0.693	0.134	0.267	0.936
Storage		0.644	0.460	0.160	0.476	0.222	0.847	0.329	0.122	0.901
Interaction		0.802	0.975	0.898	0.892	0.990	0.274	0.638	0.422	0.422

* Means of the stored treatments with and without heating were compared with the mean of the control treatment by Dunnett's test;
[a,b] Means within a row followed by different superscript letters are significantly different by Tukey's test (P < 0.05).
SEM: standard error of the mean.

The birth weight of Japanese quail is directly linked to egg weight (Grzegrzółka & Gruszczyńska 2019; Chimezie et al., 2020). Thus, the lower weight values of newly hatched chicks obtained in this study are attributed to the lower egg weight. Eggs subjected to the pre-heating process experienced the greatest weight loss, as the heat treatment protocol increased the opportunity for water evaporation from the eggs. Gharib (2013) observed in his study that broiler breeder eggs heated for nine hours during pre-storage exhibited a higher percentage of weight loss compared to those not heated. Additionally, as stated by Abdel-Halim et al. (2015), exposure of hatching eggs to long storage periods and the heat treatment process increases the opportunity for water to evaporate from the eggs.

In this study, there was a reduction in the length of newly hatched chicks when eggs were pre-heated. Goliomytis et al. (2015) found that the length of newly hatched chicks is directly related to egg weight. Therefore, in this study, this phenomenon occurred due to the greater loss of water that the fertile egg was exposed to during pre-heating. Furthermore, Lin et al. (2017) described a significant reduction in chick length and residual yolk sac weight when eggs from broiler breeders were subjected to a pre-heating temperature of 29.4 °C during storage. Kaneko et al. (2021) noted a reduction in the residual yolk sac weight of chicks from fertile broiler eggs when these were exposed to short-term temperature stimulation during incubation.

Comparing the results of heart weight in newly hatched chicks from eggs stored for 3.5, 6.5, and 9.5 days by Dunnett's test, those pre-heated showed significantly lower results than the control group ($p<0.05$). The intestinal weights of chicks from eggs stored for 9.5 days without pre-heating and eggs stored for 3.5, 6.5, and 9.5 days with pre-heating showed significantly lower results than the control group ($p<0.05$). Finally, the gizzard weight of newly hatched chicks was significantly lower than that of control group when eggs were subjected to storage (3.5, 6.5, and 9.5 days) and pre-heating ($p<0.05$). In this study, it is suggested that this result is influenced by the lower percentage of yolk sac that was evidenced in these chicks. According to Nasri et al. (2020a), this happens due to the prolonged storage of the egg, which causes a delay in the development of the yolk sac membrane, and, consequently, the development of the yolk sac is also delayed. This delay can affect the absorption capacity of the yolk sac membrane by the chick. With less yolk sac utilization, less energy is available to the developing embryo, and less energy can be deposited in organs, resulting in lower organ percentages.

Nasri et al. (2020a) conducted studies revealing that the percentage of hearts in hens was higher when eggs were stored for 5 to 12 days compared to those stored for 19 days. Additionally, chicks from fertile eggs stored for 19 days exhibited a lower percentage of intestine compared to those stored for five days (Nasri et al., 2020b). Lastly, Abioja et al. (2021) reported that the weight of the birds' gizzards was influenced by egg storage time, with heavier gizzards observed in chicks from eggs stored for 8 and 12 days and lighter gizzards in birds from fertile eggs stored for 16 days.

The productive performance of progeny from eggs subjected to the pre-heating process during the storage period (Table 4) remained unaffected by the interaction of storage and pre-heating days in phases 1 to 14 days, 15 to 35 days, and 1 to 35 days. Consequently, the days of storage and the pre-heating process had no significant effects on the weight (g), weight gain (g), feed intake (g), or feed conversion (g/g) of the birds. At an average age of 35 days, Japanese quail exhibited a weight of 138.50 g/bird, cumulative feed intake of 454.04 g/bird, and a feed conversion ratio of 3.61 g g^{-1}.

Various factors influencing the environment experienced by the embryo before laying or hatching can impact progeny development. In a study by El-Garhy et al. (2021), broiler chicks from fertile eggs subjected to a 10-day storage period and exposed to pre-heating treatment at 37.77 °C for 3.5 h exhibited significantly improved live weight and body weight gain compared to eggs stored without pre-heating.

Furthermore, Lesuisse et al. (2017) found that progeny performance in the field is influenced by variables such as the age and nutrition of laying hens, as diets provided during the laying phase directly affect progeny performance. In contrast to the present study, Duman and Şekeroğlu (2017) reported significant effects of egg weight on body weight up to seven days of chick life. Yang et al. (2021) indicated that egg storage (0 to 14 days) reduces both egg weight and chick body weight.

The results from this study highlight the need for future experiments involving pre-heating during the storage of fertile Japanese quail eggs to precisely elucidate the impacts of the protocol on egg and chick quality.

Table 4
Productive performance of Japanese quail chicks (n=20) from eggs pre-heated before incubation and stored for different periods

Variable	Control	Hours of pre-heating		Days of storage			Mean	SEM	P- value		
		0	4	3.5	6.5	9.5			Pre-heating	Storage	Interaction
Starter, 1 to 14 d											
Body weight 14 d, g	41.72	41.60	41.78	41.77	41.92	41.38	41.69	0.21	0.755	0.715	0.990
Feed intake, g	114.45	113.75	114.42	113.15	115.48	113.63	114.14	0.52	0.565	0.236	0.623
Weight gain, g	34.72	34.61	34.80	34.74	34.94	34.43	34.71	0.21	0.732	0.745	0.988
Feed conversion, g g^{-1}	3.29	3.28	3.29	3.25	3.30	3.30	3.29	0.02	0.953	0.738	0.858
Grower, 15 to 35 d											
Live weight 35 d, g	140.53	138.65	140.41	139.91	139.37	139.31	138.50	0.54	0.242	0.933	0.853
Feed intake, g	342.73	337.54	341.30	339.87	337.00	341.40	339.89	2.14	0.432	0.742	0.475
Weight gain, g	91.80	90.05	91.65	91.11	90.48	90.97	90.99	0.55	0.232	0.915	0.825
Feed conversion, g g^{-1}	3.73	3.75	3.72	3.73	3.72	3.75	3.73	0.02	0.634	0.887	0.202
Total, 1 to 35 d											
Feed intake, g	457.19	451.30	455.73	453.02	452.48	455.04	454.04	2.34	0.412	0.917	0.636
Weight gain, g	126.53	124.67	126.46	125.86	125.42	125.41	125.70	0.62	0.232	0.958	0.867
Feed conversion, g g^{-1}	3.61	3.62	3.60	3.60	3.60	3.62	3.61	0.02	0.684	0.851	0.312
Viability, %	91.25	91.25	87.50	87.50	89.37	91.25	89.64	1.33	0.199	0.564	0.085

SEM: standard error of the mean.

Conclusions

The application of pre-heating at 37.5 °C for 4 h on the third day of storage during the pre-incubation period in eggs stored for up to 9.5 days has no significant influence on egg quality, incubation performance, embryonic development, the quality of hatched chicks, or the overall progeny performance of Japanese quail.

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