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Effect of different temperatures on the incubation of European quail eggs

Efeito de diferentes temperaturas na incubação de ovos de Codornas Europeias

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Highlights

Embryonic development occurred differently according to incubation temperature.
Temperature had a direct effect on the incubation yield of European quail eggs.
Morphological quality and quail weight were affected by incubation temperature.
It was possible to identify an average temperature to incubate European quail eggs.

Abstract

Temperature is an important factor to be studied and defined in the artificial incubation of eggs, as it influences hatching success and the quality of the hatched animals. Optimal temperatures may vary depending on the species and their productive potential. In this study, we investigated the effects of various incubation temperatures on European quail eggs. A total of 1,000 eggs from two genetic groups of European quail were incubated at five different temperatures (37.0, 37.5, 38.0, 38.5, and 39.0 ± 0.2 ºC). Upon hatching, we analyzed incubation yield parameters, including incubation time, hatchability, embryodiagnosis, quail weight, and morphological quality. The results showed that temperature significantly influenced phase I of embryodiagnosis in a linear manner and had a significant quadratic effect on hatchability, phase III of embryodiagnosis, and quail weight. The average incubation time and morphological quality were also influenced by temperature. Extreme temperatures led to poorer outcomes, reducing hatchability, quail weight, and morphological quality, while increasing embryonic mortality. Incubation time decreased as the temperature increased. Intermediate temperatures of 37.9 and 38.6 ºC maximized hatchability and quail weight, respectively, with an optimal temperature range incorporating the best results for other variables: 38.1 ºC for embryonic mortality in phase 3 and 38.5 ºC for morphological quality. An average temperature

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of 38.3 ºC, between the variables hatchability and quail weight, is recommended for incubating this species.
Key words: Hatchability. Incubation. Meat-type quail. Morphological quality. Thermal optimization.

Resumo

A temperatura é um fator importante a ser estudado e definido na incubação artificial de ovos, possibilitando melhorar a eclosão e a qualidade dos animais eclodidos. As temperaturas ótimas podem variar dependendo da espécie e do potencial produtivo. Neste estudo, investigou-se os efeitos de várias temperaturas na incubação de ovos de codornas europeias. Um total de 1.000 ovos de dois grupos genéticos de codornas europeias foram distribuídos em cinco diferentes temperaturas para incubação (37,0; 37,5; 38,0; 38,5 e 39,0 ± 0,2 ºC). Após a eclosão, foram analisados parâmetros relacionados ao rendimento da incubação, como tempo de incubação, eclodibilidade, embriodiagnóstico, peso da codorna e qualidade morfológica. Os resultados mostraram que a temperatura influenciou significativamente a fase I do embriodiagnóstico de forma linear e teve um efeito quadrático significativo na eclodibilidade, fase III do embriodiagnóstico e peso da codorna. A média do tempo de incubação e da qualidade morfológica também foram influenciadas pela temperatura. Temperaturas extremas levaram a piores resultados ao reduzir a eclodibilidade, o peso das codornas e a qualidade morfológica, enquanto aumentaram a mortalidade embrionária. O tempo de incubação diminuiu conforme a temperatura aumentou. Temperaturas intermediárias de 37,9 e 38,6 °C maximizaram a eclodibilidade e o peso das codornas, respectivamente, sendo uma faixa de temperatura ideal que incorporou os melhores resultados das outras variáveis, de 38,1 ºC para mortalidade embrionária na fase 3 e 38,5 ºC para qualidade morfológica. Uma temperatura média de 38,3 ºC, entre as variáveis eclodibilidade e peso das codornas, é recomendada para incubação desta espécie.
Palavras-chave: Codorna de corte. Eclodibilidade. Incubação. Otimização térmica. Qualidade morfológica.

Introduction

The artificial incubation process for fertile eggs depends on physical factors such as temperature, humidity, turning, and ventilation (Boleli et al., 2016). These factors must be precisely regulated to ensure the full embryonic development of poultry species, thereby maximizing the quantity and quality of the hatchlings and enabling them to express their full genetic and productive potential. Embryos are vulnerable to incubation temperatures above or below the species-specific ideal (Sgavioli et al., 2015). Such deviations can lead to molecular, biochemical, and physiological changes during embryonic development, affecting bird growth both pre- and post-hatching (El-Shater et al., 2021).

In the study by Carvalho et al. (2020), exposure to 39.5 ºC for 12 h/day from day 0 to 13 during the incubation of Japanese quail eggs revealed that high temperatures impaired hatchability and negatively affected the development and physiology of the animals post-hatching. This influenced neonatal quail growth, weight, mortality, environmental heat exchange, and thermal stress tolerance compared to a control

temperature of 37.8 ºC. Another study demonstrated that incubating Japanese quail eggs at 36.0 ºC resulted in smaller and lighter birds and prolonged incubation time compared to a standard temperature of 37.5 ºC (Ben-Ezra & Burness, 2017).

There are two species of quail used for production: the Japanese quail (Coturnix coturnix japonica), primarily for egg production, and the European quail (Coturnix coturnix coturnix), specialized for meat production (Nascimento et al., 2021). European quail are earlier-developing than Japanese quail (Nascimento et al., 2021) reaching an average weight of 260.25 g at 35 days of life (Prates et al., 2023). Incubation strategies for European quail eggs often adopt temperatures established for Japanese quail, typically 37.8 ºC (Porto et al., 2021).

Studies on the optimal incubation temperature for European quail are limited. Understanding and establishing an ideal incubation temperature is crucial for improving the incubation and production management of this species. Therefore, this study aims to investigate the influence of various incubation temperatures on European quail eggs, focusing on incubation yield and defining an optimal temperature.

Material and Methods

Location, experimental population, and incubation process

The experiment was approved by the Ethics Committee on the Use of Animals at the Federal University of Minas Gerais (Belo Horizonte, MG, Brazil) on 06/12/2023, protocol number 133/2023.

The experiment was conducted in the Poultry Laboratory of the Institute of Agricultural Sciences at the Federal University of Minas Gerais, Montes Claros Campus - MG (ICA/UFMG) (16º40'40.584"S 43º50'28.428"W) between July and September 2023. The study involved 1,000 European quail hatching eggs provided by the ICA/UFMG meat-type quail genetic program comprising 96 females and 48 males from each genetic group, named ICA I and ICA II.

Eggs were collected daily for nine days, with an additional collection in the morning and night of the 9th day, totaling 10 collections. The collected eggs were selected, tagged with adhesive labels, placed in quail egg trays, and stored at room temperature, averaging 23.6 ºC, for 10 days (from day zero to nine). At the end of the storage period, the eggs were weighed and placed in five IP 130D incubators (Premium Ecológica®, Brazil), pre-set to five temperatures: 37.0, 37.5, 38.0, 38.5, and 39.0 ± 0.2 ºC. Relative humidity was maintained at 60%, and the eggs were turned every two hours. Each incubator contained eggs from all storage days and both genetic groups.

Experimental design, transfer, and birth

The experimental design was completely randomized and structured as a 5x2 factorial arrangement (five incubation temperatures and two genetic groups), with 100 replications per treatment, each egg serving as an experimental unit. On the 14th day of incubation, turning was ceased, and the eggs were candled using an ovoscope (Cold Light Ovoscope, Premium Ecológica®, Brazil) to remove clear eggs. The remaining eggs were placed individually in filo bags

and returned to the incubators until hatching. Hatchings were monitored every six hours from the emergence of the first chick, and the number of birds hatched during each interval was recorded to calculate incubation time in hours.

At hatching, the chicks were weighed and subjected to a morphological quality assessment adapted from Tona et al. (2003), in which activity, down and appearance, eyes, legs, and navel area are analyzed, with scores assigned from 0 to 60 points. The analyzed incubation yield parameters included incubation time, hatchability, and embryodiagnosis. Clear and unhatched eggs were opened to assess embryo development status according to Ainsworth et al. (2010) and classified into three stages of embryonic mortality as per Porto et al. (2021): phase I (one to seven days of embryonic development), phase II (eight to 12 days), phase III (13 to 18 days), and including phase IV (pipped but unhatched eggs).

Statistical analysis

Hatchability and embryodiagnosis data were analyzed as a function of incubation temperature using logistic regressions through the LOGISTIC procedure of SAS/STAT® [SAS Institute] (2023). By this method, 1 was defined as the probability of an event occurring and 0 as its non-occurrence. The analysis results were transformed using the logistic equation $P(x) = \frac{exp(\beta 0+\beta 1X1+\cdots \beta nXn)}{1+\exp(\beta 0+\beta 1X1+\cdots \beta nXn)}$ to generate the regression curve. The presence of outliers was checked using the PROC ROBUSTREG procedure (SAS Institute, 2023), and normality was verified through the Kolmogorov-Smirnov test (P>0.05) for morphological quality, quail weight, and incubation time. Variables with normally distributed residuals were subjected to regression analysis using the PROC REG procedure (SAS Institute, 2023), while those displaying non-parametric behavior had their means compared using the Kruskal-Wallis test at a 5% significance level. The complete model incorporated the effects of incubation temperature, genetic group, and their interaction, along with the covariates 'egg weight' and 'storage period', when significant.

Results

There was no significant difference (P>0.05) between the genetic groups or their interactions with incubation temperature across any of the analyzed variables. Incubation temperature had a significant quadratic effect on hatchability and phase III of embryodiagnosis, as well as a significant linear effect on phase I. The 'storage time' covariate significantly influenced hatchability and phase I of embryodiagnosis, while 'egg weight' had a significant effect on hatchability. No significant differences (P>0.05) were observed in any variable for phases II and IV of embryodiagnosis. There was a significant quadratic effect on quail weight, with 'egg weight' and 'storage time' as influential covariates. The highest weight, 10.03 g, was recorded at 38.6 ºC, with lower weights noted at the others temperatures (Table 1).

Table 1
Estimated hatchability rates and the different stages of embryodiagnosis and quail weight

Incubation temperature (ºC)						
Variable	37.0	37.5	38.0	38.5	39.0	P-value
Hatchability (%)	63.53	69.38	70.88	68.34	61.26	0.0293**
Phase 1 (%)	11.73	13.30	15.05	16.98	19.11	0.0375*
Phase 2 (%)	1.75	1.33	1.01	0.77	0.59	0.2707
Phase 3 (%)	16.43	10.58	8.86	9.84	14.33	0.0140**
Phase 4 (%)	8.16	7.60	7.08	6.59	6.13	0.4163
Quail weight (g)	9.83	9.94	10.00	10.02	10.01	0.0221*

Variable	Regression equation	Max/Min point on the regression curve
Hatchability	$Y= \exp -550.32+29.06x-0.383x^2/1+ \exp -550.32+29.06x-0.383x^2$	70.91% at 37.9 ºC
Phase 1	$Y= \exp -12.6709+0.2879x/1+ \exp -12.6709+0.2879x$	19.11% at 39.0 ºC
Phase 3	$Y= \exp 900.5-47.437x+0.6231x^2/1+ \exp 900.5-47.437x+0.6231x^2$	8.84% at 38.1 ºC
Quail weight	$Y= -103.7744+5.9x-0.0765x^2$	10.03 g at 38.6 ºC

** Significant linear effect.
** Significant quadratic effect.
Phase I (one to seven days of embryonic development). Phase II (eight to 12 days). Phase III (13 to 18 days). Phase IV (pipped but unhatched eggs).

Hatchability rates decreased at extreme temperatures, accompanied by an increase in mortality in phase III. In phase I, mortality increased steadily as incubation temperature rose. In phases II and IV, incubation temperature had no significant influence, as these stages are less sensitive to temperature variations. In contrast, phases I and III, which represent the beginning and end of incubation, are more critical to the incubation process. Optimal results were seen at intermediate temperatures, with a hatchability rate of 70.91% at 37.9 ºC and a mortality rate of 8.84% in phase III at 38.1 ºC.

There was a significant effect ($P<0.05$) of temperature on average incubation time and morphological quality (Table 2). Average incubation time declined as incubation temperature was increased, with eggs incubated at 37.0 ºC averaging 18.88 days, compared to 16.97 days at 39.0 ºC. The total hatch window was 6.25 days, from the emergence of the first (376 h) to the last (526 h) chick. Regarding morphological quality, lower values were observed at the lowest and highest temperatures, while 38.5 ºC resulted in the highest numerical morphological quality score.

Table 2
Mean values of incubation time and morphological quality at each incubation temperature

	Incubation temperature (ºC)					
Variable	37.0	37.5	38.0	38.5	39.0	P-value
Incubation time (h)	453.16^{a}	441.81^{b}	426.43^{c}	405.19^{d}	407.33^{d}	<0.0001
Morphological quality	48.02^{c}	51.44^{bc}	54.94^{a}	55.24^{a}	53.67^{ab}	<0.0001

$^{a-d}$ Values in rows with different letters differ significantly by the Kruskal-Wallis test at 5% probability.

Discussion

Prolonged storage time leads to a decline in egg quality, including protein disruption, albumen liquefaction, and the consequent loss of egg water to the environment. Morphological changes also occur in the blastoderm, such as cell apoptosis, which can lead to embryonic mortality (Alo et al., 2023). Therefore, eggs stored for extended periods before incubation are more likely to have already compromised embryonic development. In addition to storage time, higher incubation temperatures may increase water loss and cause thermal shock to the eggs at the time of incubation, potentially explaining the increasing linear effect of temperature on mortality in phase I (Table 1).

According to Boleli et al. (2016), water loss reduces thermal conductivity, diminishing heat exchange between the embryo and the egg surface, which in turn exchanges heat with the environment depending on the temperature differences between them. At optimal incubation temperatures, eggs acquire heat from the environment at the beginning of embryonic development. However, as the embryo develops, metabolic heat production increases its temperature; at this stage, the embryo must dissipate heat to maintain its homeostasis and ensure full embryonic development (French, 1997). Thus, eggs incubated at temperatures above the species- or genetic group-specific requirement may cause the developing embryos to experience hyperthermia, with increased heat gain in eggs through radiation and conduction. Conversely, eggs incubated at low temperatures may lose excessive heat to the environment, causing hypothermia in the embryos (Boleli et al., 2016). In both scenarios, embryonic mortality increases, reducing hatchability, which explains the quadratic effect observed in hatchability and mortality rates in phase III (Table 1).

Corroborating with Sgavioli et al. (2015), chicken eggs incubated at a thermoneutral temperature (37.5 ºC) initially gained heat and lost it toward the end of incubation, as expected. However, eggs incubated at a high temperature (39 ºC) maintained a high shell temperature throughout incubation, indicating a lack of heat dissipation. When chicken eggs were incubated at temperatures considered high (38.9 ºC) and low (36.7 ºC) relative to the thermoneutral temperature (37.8 ºC) during the incubation period (0 to 20 days), there was reduced albumen and yolk consumption, leading to a lower embryo weight, compromised nutrient absorption, and

impaired growth. This resulted in increased embryonic stress, elevated heart rate, reduced embryo movement, and ultimately, a higher mortality rate and more frequent malformations (Noiva et al., 2014).

The negative effects of extreme temperatures observed during the incubation of European quail eggs are similar to those seen with Japanese quail. Romao et al. (2009), studied eight different temperatures ranging from 34 to 41 ºC and found high mortality in phase III and low hatchability at the highest and lowest temperatures. Optimal hatchability results, 76.67% and 80.76%, were achieved at 37 and 38 ºC respectively. In contrast, temperatures as low as 34 ºC prevented any animal from hatching. Porto et al. (2021) also found that higher incubation temperatures, specifically 39.5 ºC, resulted in lower hatchability rates and increased total embryonic mortality, whereas 38.5 ºC yielded better hatchability rates for Japanese quail eggs. Moreover, higher temperatures led to reduced nutrient utilization in the embryonic development process, contributing to increased embryonic mortality and decreased hatchability (Abuoghaba et al., 2021).

Incubation temperatures influenced embryo metabolism, affecting incubation times. Eggs incubated at lower temperatures required more time to hatch compared to those at higher temperatures. Ben-Ezra and Burness (2017) noted that Japanese quail eggs incubated at 36 ºC took approximately 18.5 days to hatch, whereas those at 37.5 ºC hatched in 16 days. Alongside prolonged incubation times, lower temperatures slowed embryonic development, increased yolk consumption, and heightened energy expenditure during the incubation process, leaving less residual energy at hatching (Wada et al., 2015). These factors negatively impacted overall embryonic development, ultimately reducing quail weight and quality at birth, which could increase mortality and lower hatchability. This was observed in the present study and is supported by findings in wood ducks (Aix sponsa), where incubation temperatures of 35 and 35.9 ºC increased energy expenditure by 20% and 37%, respectively, compared to the highest temperature of 37 ºC (Durant et al., 2011). The authors attributed this increased energy expenditure to the extended incubation times necessitated by lower temperatures, with hatching delayed by 1.2 to 1.9 days.

An incubation temperature of 38.6 ºC was observed to maximize quail weight. Similarly, Nord and Nilsson (2021) reported an effect of incubation temperature on Japanese quail weight, with weights of 8.59 g at 38.5 ºC, 8.15 g at 37 ºC, and 7.46 g at 35.5 ºC, with the greatest weight recorded at 38.5 ºC and the lowest at the minimum temperature. Regarding morphological quality, Karakelle et al. (2023) described lower scores, reaching 99 points, when exposing Japanese quail eggs to hypothermia stress at 35 ºC for 6 h/day from the 9th to the 15th day of incubation. In contrast, eggs incubated at 37.5 ºC until the 15th day produced chicks of superior quality, scoring 99.46 points, corroborating our findings, where lower temperatures reduced the morphological quality of the chicks.

Incubation at high temperatures also causes water loss from the eggs, accelerates embryonic development, and increases embryo metabolism, shortening incubation times and resulting in low birth weight and poor animal quality among hatchlings (Abuoghaba et al., 2021; Sgavioli et al., 2015). Similarly,

when chicken eggs were incubated under thermal manipulation at 39.6 ºC, the resultant chick quality at birth was poor (Narinç et al., 2016). The low birth weight stemmed from inadequate nutrient absorption from the yolk during the shortened incubation process. Molenaar et al. (2010, 2011) observed that chicks incubated at a high temperature (38.9 ºC) relative to the control temperature (37.8 ºC) were smaller, had less body weight without the yolk, retained a greater quantity of yolk, and exhibited reduced protein utilization. A lower hepatic glycogen level available for the hatching process was also noted, potentially necessitating the use of glucogenic amino acids, thereby diminishing their availability for muscle formation and body development.

As observed in this study, the effects of high and low temperatures from the start of incubation trigger chain reactions in the embryonic development process, creating challenges in energy utilization, embryonic development, survival, and overall animal quality. As observed by Ipek et al. (2014) in different temperature ranges when incubating chicken eggs (33.3 to 36.7, 37.8 to 38.2, and 38.9 to 40 ºC), both low and high temperatures led to poorer outcomes in incubation parameters, increased embryo mortality, and reduced hatchability, weight, and animal quality, ultimately decreasing the number of viable hatchlings. Moreover, incubation times decreased at high temperatures and increased at low temperatures.

Conclusion

In conclusion, incubation at temperatures outside the ideal range leads to modulations in metabolism and embryonic development, culminating in decreased hatchability, weight, and quality of European quail. Specifically, a temperature of 37.9 ºC was found to maximize hatchability, whereas a temperature of 38.6 ºC maximized quail weight. Thus, an average temperature of 38.3 ºC is recommended for incubating European quail eggs to enhance morphological quality, quail weight, and hatchability rate. Future experiments should consider other physical factors related to egg incubation that also contribute to optimal incubation yield, such as relative humidity and egg turning frequency, which have not yet been examined in European quail.

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