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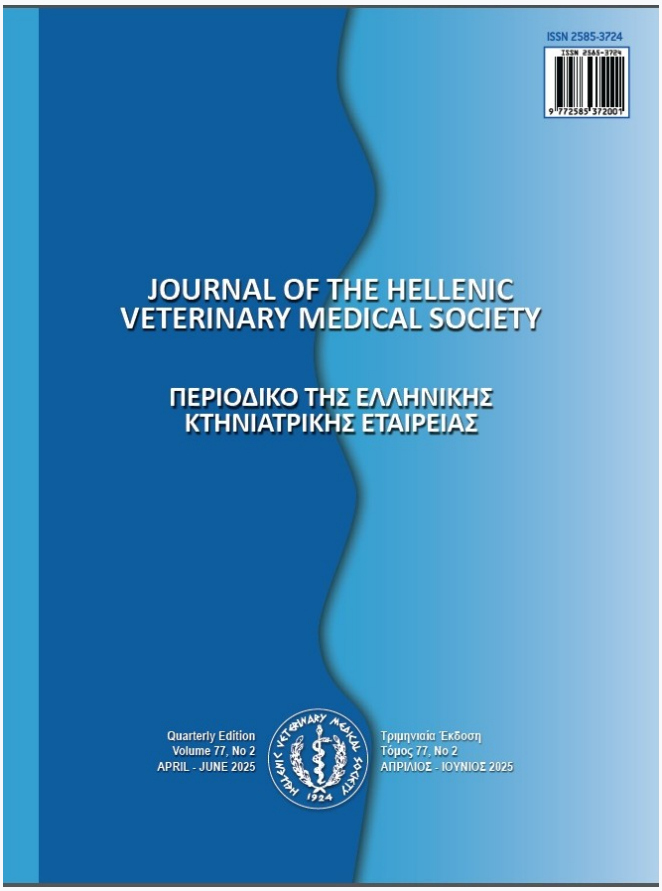

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Research article
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Effect of Thermal Manipulations during Incubation on Hatching results and chick sex ratio of layer breeder eggs

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ABSTRACT: The study evaluated the effects of different setter temperature applications (TA) during early (3-6 days) and late (16-18 days) incubation periods on embryonic mortality, hatchability, chick quality, and male-female chick ratio of eggs from Atak-S (experiment 1) and Isa Tinted (experiment 2) parent stock breeder flocks. Each experiment used a total of 1050 fertile eggs, which were randomly divided into 5 experimental groups. Group 1 served as the control (CE) with standard incubation at an average temperature of 37.6°C (99.68 °F) and 55% RH throughout the first 18 days (432 hours) of incubation (DOI). Group 2 (Early period low - EPL) experienced eggs were incubated at 36.6°C (97.88 °F) and intermittent temperature increases to 36.8°C (98.24 °F) and 37°C (98.60 °F) at 3 and 6 DOI for 2 hours. Group 3 (Early period high - EPH) eggs were incubated at 38.1°C (100.58 °F) and intermittent temperature increases to 38.3°C (100.94 °F) and 38.5°C (101.30 °F) at 3 and 6 DOI for 2 hours. Group 4 (Late period low - LPL) experienced eggs were incubated at 36.6°C and intermittent temperature increases to 36.8°C and 37°C at 16 and 18 DOI for 2 hours. Finally, group 5 (Late period high-LPH) eggs were incubated at 38.1°C and intermittent temperature increases to 38.3°C and 38.5°C at 16 and 18 DOI for 2 hours. The results revealed significant effects of temperature treatments on hatch of fertility (HOF) and gender ratios among treatment groups in both trials ($P<0.05$). In experiment 1, all TA treatment groups exhibited a high female-chick ratio, with EPL-treated eggs having the highest female-chick rate (61.61%). In experiment 2, LPH-treated eggs showed the highest female-chick ratio (54.9%). All TA treatments improved chick quality, as determined by the Tona score ($P<0.001$). Chicks hatched from LPL and LPH showed the highest Tona chick quality score in both trials. In conclusion, exposing eggs to low temperatures during the early (EPL) (3 and 6 days) period or to high temperatures during the late (LPH) (16 and 18 days) period of incubation could effectively increase female chick rates at hatch due to an increase in embryonic male mortality.

Keyword: canine; Atak-S; IsaTinted; Temperature; Hatchability; sex ratio.

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INTRODUCTION

In egg industry male chicks are not important because males have no economic interest and they do not lay eggs and grow more slowly than broilers fast-growing optimized to produce a lot of meat in record time (slaughter weight reached in 42 days) (Guillaume, 2016). In world an estimated 4-6 billion male chicks are killed globally every year because they serve no economic purpose (Chase, 2018). In Germany, Europe's third-largest egg producer, 45 million male chicks are slaughtered each year, most often crushed or gassed (Delphine, 2019). In order to remedy this, the German government has just banned the crushing of male chicks, imposing a technically mastered alternative, spectrometry (Clément, 2021). Two methods are currently the subject of extensive research; the Raman spectroscopy method (3 days incubation, invasive), developed in Germany by the University of Leipzig. The other method, which is done by detecting sex indicator substances (a priori non-invasive), is being developed in the Netherlands and France which the sex is detected at 9 days of incubation (Nicolas, 2019).

The temperature during incubation can play an important role in embryo sex determination. In fish, reptiles, and amphibians, sex determination is affected by some external factors such as temperature which play the most important role in sex determination during incubation (Ferguson and Joanen, 1982; Deeming and Ferguson, 1989; Strussmann and Patino, 1995; Wallace et al., 1999; Selim et al., 2009).

In this perspective to link sex determination with temperature numerous studies have been carried out but no convincing evidence for temperature-depending sex determination was found (Pike and Petrie, 2003). However, recent previous studies reported that incubation temperature can have an influence on the chick sex ratio (Ferguson's 1994; Göth and Booth, 2005; Tzschentke and Halle, 2009, 2010; Halle et al., 2012; Yilmaz et al., 2011). Additionally, it has been reported that in domestic chickens during hatching the proportion of male chicks is almost equal to that of female chicks (Lambert and Knox, 1926).

Research on sex determination in domestic chickens has shown that primary sex determination begins at fertilization within the infundibulum and becomes functionally active during the initial days of incubation, specifically manifesting between days 6.5-8.5. However, the influence of temperature variations during early (days 3-6) and late (days 16-18) incubation periods on sex ratio, hatchability, and chick quality remains unclear and understudied.

This study hypothesizes that controlled thermal manipulation during these critical developmental windows could potentially shift sex ratios, enhance hatchability rates, and improve chick quality in layer breeds. By exploring these variables, we aim to provide novel insights into how incubation conditions can modulate developmental processes in avian embryos. Unlike previous research, this study not only seeks to validate existing findings but also to uncover new implications of incubation temperatures on chick characteristics, thus contributing a fresh perspective to the field.

MATERIALS AND METHODS

Ethics statement

Criteria specified by European policy for the protection of animals (European Union 2010) were followed during experimental period.

Study site and breeder flock

The experiment was conducted at the incubation laboratory in the Department of Animal Science, Faculty of Agriculture, Selcuk University. Two independent experiments were carried out using eggs obtained from 48-week-old Atak-S and 40-week-old Isa Tinted parent stock breeder flocks for experiment 1 and experiment 2, respectively.

Egg collection and experimental design

A total of 1050 eggs were collected on the same day for both experiments. The eggs were placed in carton trays and transported to the incubation laboratory at Selcuk University. In current study thermal manipulations were applied at early (3-6 d) and late period (16-18d) of incubation at low (36.6-36.8 -37 °C, 2h) and high (38.1-38.3-38.5°C, 2h) incubation temperature not at 1-17 d of incubation as described by Tzschentke and Halle (2009), who exposure eggs from d 1 to 17 under normal incubation conditions (37.2-37.4°C) and then sorted into three hatch incubators (control: 37.2-37.4°C; chronic warm incubation: 38.2-38.4 °C, 24 h daily; short-term warm stimulation: 38.2-38.4 °C, 2 h daily). The eggs were then randomly divided into 5 groups, with each group containing 210 eggs (divided into three egg trays of 70 eggs in each treatment group) as follows:

1. Control eggs (CE) were incubated at an average temperature of 37.6°C (99.68 °F) and 55% relative humidity (RH) without any extra heat

treatment during the first 18 days of incubation.

2. Early period low (EPL) temperature treatment group, in which the eggs were incubated at 36.6°C (97.88 °F) and intermittently increased to 36.8°C (98.24° F) and 37°C (98.60 °F) at 3 and 6 days of incubation for two hours before being returned to the standard incubation profile.
3. Early period high (EPH) temperature treatment group, in which the eggs were incubated at 38.1°C (100.58 °F) and intermittently increased to 38.3°C (100.94 °F) and 38.5°C (101.30 °F) at 3 and 6 days of incubation for two hours before being returned to the standard incubation profile.
4. Late period low (LPL) temperature treatment group, in which the eggs were incubated at 36.6°C (97.88 F) and intermittently increased to 36.8°C (98.24 °F) and 37°C (98.60 °F) at 16 and 18 days of incubation for two hours before being returned to the standard incubation profile.
5. Late period high (LPH) temperature treatment group, in which the eggs were incubated at 38.1°C (100.58 °F) and intermittently increased to 38.3°C (100.94 °F) and 38.5°C (101.30 °F) at 16 and 18 days of incubation for two hours.

All treatment group eggs were incubated together in the same setter, except during the treatment days. Two identical setters were used during the study. The first setter followed the standard incubation profile, while the second one was used for the treatment applications, adjusted according to a special study plan (day/time). After the heat treatment, the eggs were returned to the same setter profile (setter 1).

Hatching result and sexing

Upon hatching, the hatchability of fertile eggs (HOF) was determined, and all hatched chicks were sorted and sexed. Unhatched eggs were carefully broken open to examine the different stages of embryonic mortality. The following stages were used to determine the period in which embryonic death occurred: early stage from day 3 to 7 (characterized by very visible blood vessels), middle stage from day 8 to 14 (feather tracts visible, upper and lower beak equal in length), and late stage from day 15 to 21 (large embryo with the egg yolk sac outside). For late mortality embryos, necropsies were performed to determine their sex. Male chick embryos were identified by the presence of two testes (on the right), while female chick embryos were recognized by having only one ovary (on the left) (figure 1).

Chick quality

After hatching, a total of 40 randomly selected female chicks from each group were examined macroscopically to assess their quality criteria. These chicks were chosen at random to represent different characteristics associated with first or second-grade quality. The determination of quality criteria was based on various physical attributes of day-old chicks, including their activity level, feathering, eye appearance, leg conformation, navel area aspect, and yolk absorption (Tona et al., 2003). The quality score

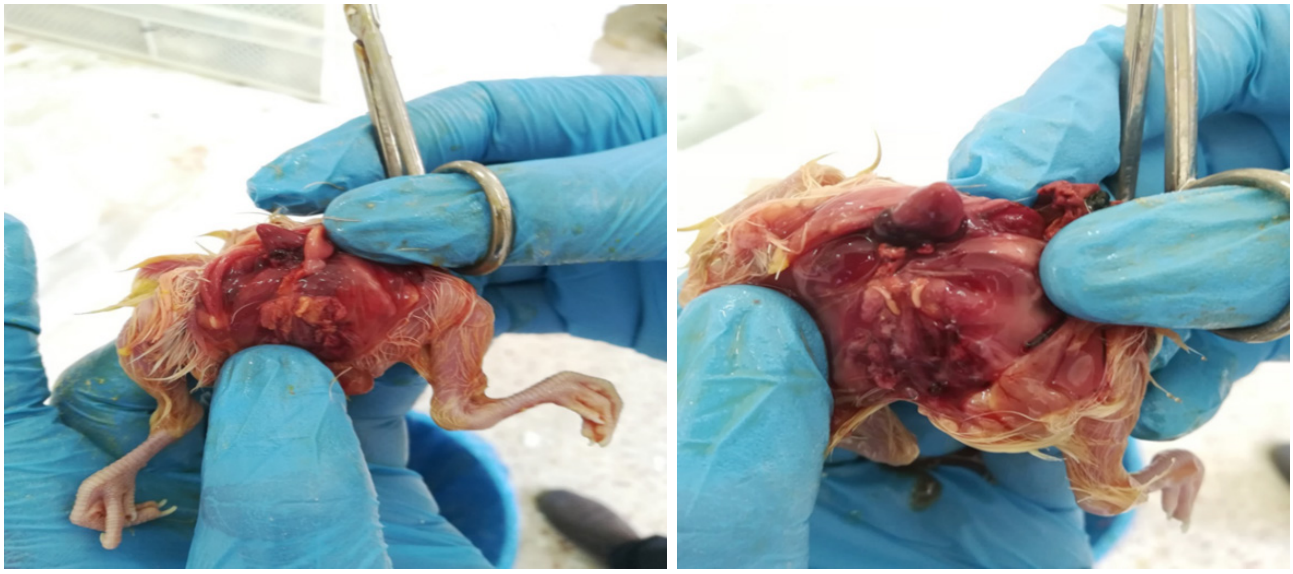


Figure 1. Late unhatched embryo sex determination.

for each chick was calculated as the sum of scores assigned to all the observed characteristics (Table 1).

STATISTICAL ANALYSIS

The data were analyzed using one-way ANOVA with the GLM procedure in SAS (SAS, 2004). The statistical model for analyzing the hatchability of fertile eggs, embryonic mortalities, sex ratio, and chick quality by Tona score was expressed as $Y_{ij} = \mu + TM_i + e_{ij}$, where Y_{ij} represented the dependent variable, μ was the overall mean, TM_i represented the Thermal manipulation (i = EPL or EPH or LPH or LPL), and e_{ij} was the is the residual error term. Differences among the treatment means were determined using Tukey's multiple range test. Claims of significant difference were based upon $P < 0.05$.

RESULTS

Hatchability characteristics

Table 2 presents the effect of thermal manipulation during incubation on hatching results. Thermal manipulation significantly affected hatchability of fertile eggs (HOF) and embryonic mortality (EM) stages ($P<0.001$). In experiment 1, the LPL eggs exhibited the highest HOF, while the LPH eggs had the lowest HOF. However, no significant differences were observed among the other treatment groups (CE, LPL, and LPH). Among the thermal manipulation (TM) treatment groups, the LPH eggs showed the highest early EM ($P<0.001$), similar to the CE group. Additionally, late EM was higher in all TM-treated eggs compared to the CE group ($P<0.05$), except for the LPL group, which did not show a significant dif-

Table 1. Criteria for determining chick quality in Tona score method

Parameters	Characteristics	Scores
Activity	Good	6
	Weak	0
Downs and appearance	Clean and dry	10
	Wet	8
	Dirty and wet	0
Retracted yolk	Body with normal swallowed yolk	12
	Body with swallowed large yolk and rather hard to touch	0
Eyes	Opened and bright	16
	Opened and not bright	8
	Closed eyes	0
Legs	Normal legs and toes	16
	One infected leg	8
	Two infected legs	0
Navel	Completely closed and clean	12
	Not completely closed and not discolored	6
	Not closed and discolored	0
Remaining membrane	No membrane	12
	Small membrane	8
	Large membrane	4
	Very large membrane	0
Remaining yolk	No yolk	16
	Small yolk	12
	Large yolk	8
	Very large yolk	0

Adapted from Tona et al. (2003).

Table 2. The effect of temperature application on hatchability of fertile eggs, early, middle and late embryonic mortality

Group		Embryonic mortality		
Experiment 1	**HOF**	**Early (3-7)**	**Middle (8-14)**	**Late (15-21)**
Atak-S parent stock flocks' eggs				
CE	91.29^{ab}	3.78^{a}	1.70	2.13^{b}
EPL	91.44^{ab}	2.53^{b}	0.99	3.53^{a}
EPH	91.50^{ab}	2.50^{b}	1.06	3.49^{a}
LPL	93.17^{a}	2.14^{b}	1.03	3.12^{ab}
LPH	89.93^{b}	4.52^{a}	0.99	4.01^{a}
SEM	0.56	0.29	0.20	0.34
P-value	0.002	0.000	0.067	0.002
Experiment 2				
Isa Tinted parent stock flocks' eggs				
CE	80.57^{a}	3.54^{b}	6.10^{a}	5.15^{b}
EPL	74.63^{b}	7.12^{a}	3.29^{b}	9.52^{a}
EPH	80.54^{a}	6.75^{a}	2.18^{b}	6.98^{ab}
LPL	81.41^{a}	5.27^{ab}	3.91^{b}	7.88^{ab}
LPH	80.58^{a}	4.99^{ab}	2.23^{b}	8.96^{a}
SEM	1.32	0.77	0.55	0.88
P-value	0.002	0.008	0.000	0.005

$^{a-b}$ Means within a column with no common superscript letter are significantly different ($P < 0.05$),

ference. There were no statistical differences among all treatment groups in terms of Middle EM.

In experiment 2, HOF in EPL eggs was the lowest compared to other treatment groups, which exhibited statistically similar HOF ($P<0.05$). Early EM was higher in EPL and EPH treatment groups compared to CE-treated eggs ($P<0.05$). However, the difference was statistically similar to LPL and LPH-treated eggs. The average middle EM was highest in CE-treated eggs compared to TM treatment eggs ($P<0.001$). Late EM was higher in EPL and LPH-treated eggs when compared to the CE group ($P<0.05$), and intermediate to EPH and LPL-treated eggs.

CE: Control eggs were incubated at an average temperature 37.6 °C (99.68°F) and 55 % RH without any extra heat treatment during the first 18 d of incubation; EPL= Early period low temperature treatment group eggs were incubated at 36.6°C (97.88 °F) and then were intermittently increased to 36.8°C (98.24°F) and 37°C (98.6 °F) at 3 and 6 days of incubation during 2 hours; ; EPH: Early period high eggs were incubated at 38.1°C (100.58 °F) and then were intermittent increased to 38.3°C (100.94° F) and 38.5°C (101.30 °F) at 3 and 6 days of incubation during two hours ; LPL: Late period low eggs were incubated at 36.6°C (97.88 °F) and then were intermittently increased to 36.8°C (98.24°F) and 37°C (98.6 °F) at 3 and 6 days of incubation during 2 hours and LPH: Late period high eggs were incubated at 38.1°C (100.58 °F) and then were intermittently increased to 38.3°C (100.94 °F) and 38.5°C (101.30 °F) at16 and 18 days of incubation during 2 hours, HOF: hatch of fertile eggs.

Late embryo mortality gender

Table 3 displays the influence of thermal manipulation during incubation on late embryonic mortality gender. In experiment 1, although the difference was not statistically significant, EPL and LPH eggs showed numerically higher late female EM compared to the other treatment groups, while the late male EM was the lowest in CE eggs ($P=0.602$; $P=0.131$). In experiment 2, the late female EM was higher in EPH, LPL, and LPH compared to EPL, which exhibited the lowest value ($P<0.001$) and was intermediate to CE-treated eggs. On the other hand, the late male EM was higher in EPL eggs compared to CE, EPH, and LPL eggs ($P<0.001$), and it was intermediate to LPH.

Table 3. The effect of temperature application on late embryonic mortality sex ratio

	Late Embryonic Mortality			
	Experiment 1		Experiment 2	
	Atak-S parent stock flocks' eggs		Isa Tinted parent stock flocks' eggs	
Group	Female	Male	Female	Male
CE	1.61	0.51	2.52^{ab}	2.64^{b}
EPL	2.05	1.47	0.51^{b}	8.50^{a}
EPH	1.54	1.96	3.59^{a}	3.39^{b}
LPL	1.51	1.62	3.31^{a}	4.57^{b}
LPH	2.53	1.47	3.36^{a}	5.61^{ab}
SEM	0.53	0.40	0.57	0.86
P-value	0.602	0.131	0.001	<0.001

$^{a-b}$ Means within a column with no common superscript letter are significantly different (P < 0.05),

CE: Control eggs were incubated at an average temperature 37.6 °C (99.68°F) and 55 % RH without any extra heat treatment during the first 18 d of incubation; EPL= Early period low temperature treatment group eggs were incubated at 36.6°C (97.88 °F) and then were intermittently increased to 36.8°C (98.24°F) and 37°C (98.6 °F) at 3 and 6 days of incubation during 2 hours; ; EPH: Early period high eggs were incubated at 38.1°C (100.58 F) and then were intermittent increased to 38.3°C (100.94 °F) and 38.5°C (101.30 °F) at 3 and 6 days of incubation during two hours ; LPL: Late period low eggs were incubated at 36.6°C (97.88 °F) and then were intermittently increased to 36.8°C (98.24 ° F) and 37°C (98.6 F) at 3 and 6 days of incubation during 2 hours and LPH: Late period high eggs were incubated at 38.1°C (100.58° F) and then were intermittently increased to 38.3°C (100.94 °F) and 38.5°C (101.30 °F) at16 and 18 days of incubation during 2 hours.

Chick sex ratio

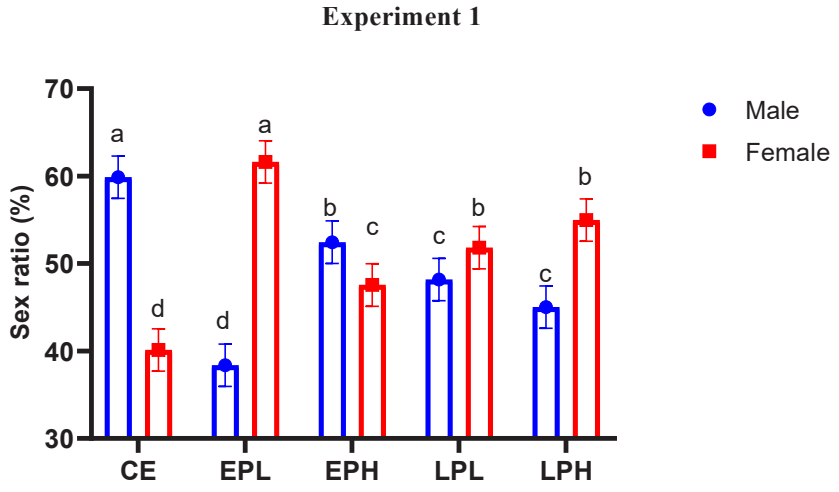
Figure 2 illustrates the impact of TM treatment on the chick gender ratio. The presented results indicate a significant effect of thermal manipulation on the female and male chick ratio (P<0.05). In experiment 1, the EPL-treated eggs exhibited the highest percentage of female chicks (61.61%) and the lowest male-chick ratio (38.39%), compared to CE-treated eggs which showed the lowest percentage of female chicks (40.11%) and the highest male-chick ratio (59.89%). However, all TM-treated eggs, except for EPH (47.55% female and 52.45% male) treated eggs, exhibited a higher female-chick ratio (LPL=51.81% female and 48.19% male; LPH= 54.99% female and 45.01% male).

In experiment 2, the LPH-treated eggs exhibited the highest percentage of female chicks (50.53%) and the lowest male-chick ratio (48.47%) compared to LPL-treated eggs (45.64% female and 54.36% male). However, although LPH eggs showed the highest percentage of female chicks, no statistically significant differences were found among CE, EPL, and EPH-treated eggs (P>0.05).

CE: Control eggs were incubated at an average temperature 37.6 °C (99.68°F) and 55 % RH without any extra heat treatment during the first 18 d of incubation; EPL= Early period low temperature treatment group eggs were incubated at 36.6°C (97.88° F) and then were intermittently increased to 36.8°C (98.24°F) and 37°C (98.60° F) at 3 and 6 days of incubation during 2 hours; ; EPH: Early period high eggs were incubated at 38.1°C (100.58 °F) and then were intermittent increased to 38.3°C (100.94 °F) and 38.5°C (101.30 °F) at 3 and 6 days of incubation during two hours ; LPL: Late period low eggs were incubated at 36.6°C (97.88 °F) and then were intermittently increased to 36.8°C (98.24°F) and 37°C (98.60 °F) at 3 and 6 days of incubation during 2 hours and LPH: Late period high eggs were incubated at 38.1°C (100.58 F) and then were intermittently increased to 38.3°C (100.94 °F) and 38.5°C (101.30° F) at16 and 18 days of incubation during 2 hours

Chick quality

Figure 3 presents the evaluation of chick quality using the methodology of Tona et al. (2003). In



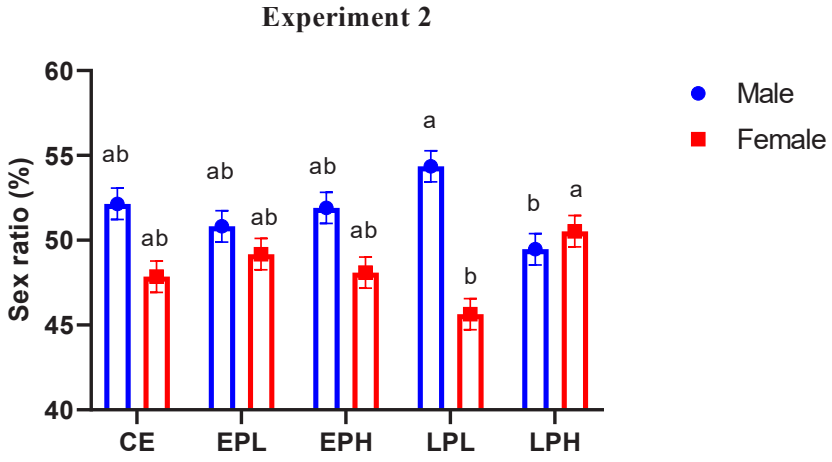


Figure 2. The effect of thermal manipulations on sex ratio.
a–b Means within a column with no common superscript letter are significantly different ($P < 0.05$),

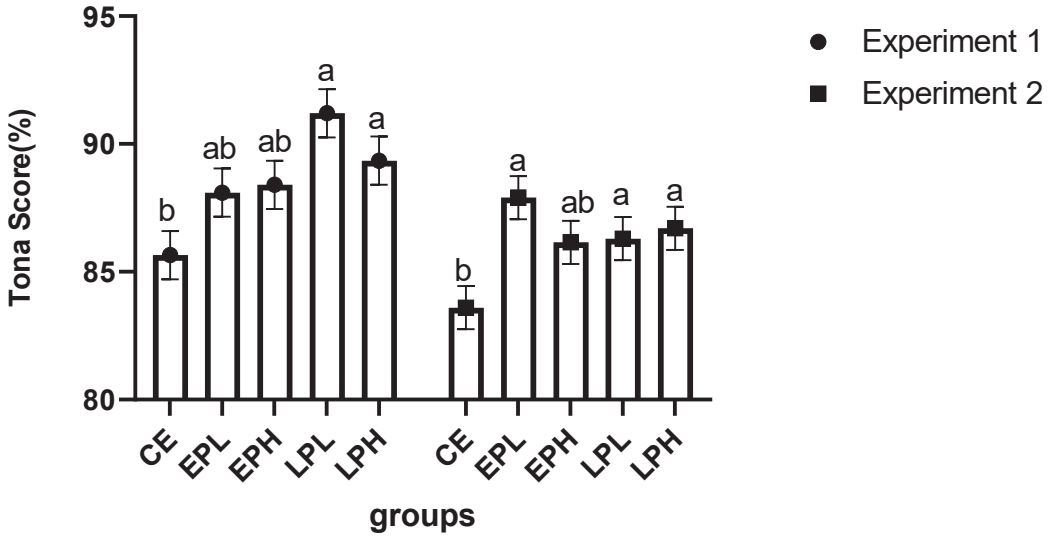


Figure 3. The effect of thermal manipulations on Tona score chick quality
a–b Means within a column with no common superscript letter are significantly different ($P < 0.05$),

both experiments, thermal manipulation (TM) significantly affected the chick quality score ($P<0.05$). In experiment 1, chicks hatched from LPL (91.20) and LPH (89.35) exhibited better Tona chick quality compared to those hatched from CE-treated eggs (85.65) ($P<0.001$). Similarly, in experiment 2, chicks hatched from EPL (87.90), LPL (86.30), and LPH (86.70) showed superior Tona chick quality than those hatched from CE-treated eggs (83.60) ($P<0.001$). However, the difference was intermediate with chicks hatched from other TM treatment group eggs in both trials ($P<0.05$).

CE: Control eggs were incubated at an average temperature 37.6 °C (99.68°F) and 55 % RH without any extra heat treatment during the first 18 d of incubation; EPL= Early period low temperature treatment group eggs were incubated at 36.6°C (97.88 °F) and then were intermittently increased to 36.8°C (98.24°F) and 37°C (98.60 °F) at 3 and 6 days of incubation during 2 hours; ; EPH: Early period high eggs were incubated at 38.1°C (100.58 °F) and then were intermittent increased to 38.3°C (100.94 °F) and 38.5°C (101.30 °F) at 3 and 6 days of incubation during two hours ; LPL: Late period low eggs were incubated at 36.6°C (97.88 °F) and then were intermittently increased to 36.8°C (98.24°F) and 37°C (98.60 °F) at 3 and 6 days of incubation during 2 hours and LPH: Late period high eggs were incubated at 38.1°C (100.58 °F) and then were intermittently increased to 38.3°C (100.94 °F) and 38.5°C (101.30 °F) at16 and 18 days of incubation during 2 hours

DISCUSSION

In the current study, applying different temperatures during early (3-6 days) and late (16-18 days) incubation periods significantly influenced hatchability, embryonic mortality rates, and chick quality. Specifically, high and low setter temperatures during these critical developmental windows led to decreased hatchability and increased embryonic mortality in both trials. These findings align with previous research, including Lourens et al. (2005), who reported that high setter temperatures during the later stages of incubation increased late embryonic mortality, reducing the hatchability of viable, first-grade chicks. In current study, the decrease in hatchability of fertile eggs in the LPH treatment group can be explained by the fact that at the late period, the embryo produces metabolic heat, and then at this period, exposure to high temperature caused a high late embryonic mortality. Notably, the manipulation of incubation temperatures also had a discernible impact on the sex ratio of hatched chicks. Lower setter temperatures during the early incubation period (3-6 days) or higher temperatures during the late period (16-18 days) led to a higher proportion of female chicks, likely due to increased male embryo mortality. This observation supports findings by Ferguson (1994), who noted that temperature fluctuations could induce sex reversal, and by Göth and Booth (2005), who demonstrated that specific temperatures could alter sex ratios in Australian brush turkeys. The differential susceptibility of male and female embryos to temperature may stem from physiological differences in how each sex responds to thermal stress. Studies, such as those by Tzschentke and Halle (2009, 2010), have indicated that male embryos are particularly vulnerable to elevated incubation temperatures, leading to a higher incidence of male mortality at these temperatures. Conversely, lower incubation temperatures have been associated with increased mortality among female embryos (Durant et al., 2016). This sensitivity may be due to the varying energy demands and metabolic rates of male and female embryos, with males potentially being more affected by hyper thermic conditions. A likely mechanism for this temperature-driven sex discrimination could involve thermal influences on gene expression related to sex differentiation pathways, which might activate or suppress genes differently in male and female embryos. Additionally, as observed by Göth and Booth (2005) and Halle et al. (2012), temperature influences hormonal environments during incubation, which may lead to differences in embryo development that manifest as increased mortality in one sex over the other. Our findings in male and female embryonic mortalities can be explained by the reasons given by Khamoun et al. (2024) that increasing the incubator temperature to 38 ◦C, whether applied throughout the entire incubation period or during specific periods (days 3–6), led to genetic females (with W chromosomes) developing testes, indicating a transition into gonadal males. Conversely, lowering the incubator temperature to 36 ◦C during days 3–6 resulted in genetic males developing ovaries, indicating a transition into gonadal females.

In our study, temperature manipulation (TA) positively affected chick quality as measured by the Tona score, particularly in chicks from eggs exposed to high temperatures during the late incubation periods (16-18 days). The better Tona scores observed in thermally manipulated chicks could be due to the

adaptive stress response to controlled temperature increases, which may enhance metabolic efficiency and overall robustness in chicks. These results are consistent with findings by El-Zeniny et al. (2019) and Joseph et al. (2006), who reported improved chick quality in groups subjected to thermal manipulation. Interestingly, the Hatchability of Fertile Eggs (HOF) was notably lower in the Late Period High (LPH) temperature group for Atak-S parent stock eggs. This reduction could be attributed to the heightened sensitivity of embryos to elevated temperatures during late-stage incubation, a phase critical for final physiological adjustments before hatching. During this period, embryos undergo significant metabolic changes, making them particularly susceptible to external stressors, such as high temperatures. Previous studies have similarly indicated that late-stage high temperatures can increase embryonic mortality due to overheating, disrupting normal developmental and metabolic processes (Lourens et al., 2005; Tzschentke & Halle, 2009). Moraes et al. (2004) also found that heat conditioning in the late incubation stages could delay hatching, potentially leading to developmental issues and increased mortality. These findings emphasize the importance of precise temperature management, especially in the late stages of incubation, to optimize hatchability and reduce embryonic losses.

The results of our study suggest that temperature adjustments during specific incubation periods not only affect hatchability and chick quality but also play a significant role in shaping the sex ratio of hatchlings. These findings underscore the need for further investigation into temperature thresholds and the physiological mechanisms underlying thermal sensitivity differences between male and female embryos. Such research could inform optimal incubation protocols to manage sex ratios, particularly in layer breeds where female-biased ratios are economically favorable. Additionally, understanding the optimal temperature conditions could help improve overall chick quality, enhancing productivity in commercial poultry production systems.

CONCLUSION

The application of controlled thermal manipulation, utilizing both low (36.6–37℃) and high (38.1–38.5℃) setter temperatures during early (3-6 days) and late (16-18 days) incubation periods, demonstrated no adverse effects on overall hatchability, embryonic mortality, or chick quality in the present study. Notably, these temperature treatments yielded a significant increase in the proportion of female chicks hatched from eggs of 48-week-old Atak-S and 40-week-old Isa Tinted breeder flocks, suggesting that thermal conditions during incubation may influence sex-specific embryo viability.

Furthermore, exposure to low and high incubation temperatures during early and late stages, respectively, resulted in marked improvements in chick quality, as indicated by enhanced Tona scores in both Atak-S and Isa Tinted flocks. These findings align with emerging research suggesting that precise thermal regulation may not only enhance hatch outcomes but also play a role in modulating offspring sex ratios through differential male and female embryonic survival under specific temperature conditions.

This study highlights the potential of targeted thermal manipulation as a tool for optimizing both hatchability and sex ratio management in commercial breeder operations. However, further research is warranted to establish whether these observed effects on sex ratios and chick quality are consistent across various parent stock lines and to elucidate the physiological mechanisms underpinning temperature-dependent sex bias in avian embryos.

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CONFLICT OF INTEREST

No potential conflict of interest was reported by the authors.

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