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Effects of thermal manipulation and photoperiodic lighting during incubation on hatching performance, hatching time, chick quality and organ growth*

Kuluçka sırasında sıcağa alıştırma ve fotoperiyodik aydınlatmanın kuluçka performansı, kuluçka süresi, civciv kalitesi ve organ gelişimi üzerindeki etkisi

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ABSTRACT

Objective: The objective of this study was to investigate the effects of thermal manipulation (TM) and photoperiodic lighting during the incubation on hatching performance, hatching time, chick quality and organ growth.

Material and Methods: The study was composed of two consecutive experiments. The 1st experiment was application of TM, 1ºC higher eggshell temperature (EST, 38.8ºC) for 6 h/day from 11 to 16 embryonic day, or optimal EST of 37.8ºC (Control) during the incubation. In second experiment, eggs were exposed to a photoperiodic lighting of 16h light and 8h darkness (16L: 8D) along with optimal EST (Light-Control) or TM (Light-Heated).

Results: Thermal manipulation accelerated hatching time, reduced chick quality score, decreased relative bursa and tibia weights while heart and sternum weights increased with no effect on hatching performance. However, 16L: 8D photoperiodic lighting schedule along with TM significantly improved chick length at hatch indicating improved chick quality while other traits were not affected except for higher relative liver weight.

Conclusion: It can be concluded that TM alone had negative effects on chick quality. However, photoperiodic lighting along with TM can be a positive approach towards better chick quality and post hatch performance as indicated by increased chick length and liver weight.

Keywords: Chick quality, hatching performance, incubation lighting, thermal manipulation and egg shell temperature

ÖZ

Amaç: Bu çalışmanın amacı, kuluçka sırasında sıcağa alıştırma ve fotoperiyodik aydınlatmanın kuluçka performansı, kuluçka süresi, civciv kalitesi ve organ büyümesi üzerindeki etkisini araştırmaktır.

Materyal ve Yöntem: Çalışma birbirini takip eden iki denemeden oluşmuştur. Birinci denemede, kuluçka optimal kabuk sıcaklığı (37.8ºC, Kontrol) veya kuluçkanın 11-16. günleri arasında günde 6 saat optimumdan 1ºC daha yüksek kabuğu sıcaklığı (38.8ºC) kullanılarak sıcağa alıştırma yapılmıştır. İkinci denemede, yumurtalara 16 saat aydınlık 8 saat karanlık (16A: 8K) aydınlatma altında optimum sıcaklık veya aydınlatma ile birlikte sıcağa alıştırma (38.8ºC) uygulanmıştır.

Araştırma Bulguları: Sıcağa alıştırma kuluçka perfromansını etkilememiş ancak kuluçkadan çıkışı hızlandırmış, civciv kalitesi, bursa ve tibia ağırlıklarını geriletirken kalp ve sternum ağırlıklarını artırmıştır. İkinci denemede sıcağa alıştırma ile birlikte 16A: 8K aydınlatma uygulanması civciv uzunluğu ve karaciğer ağırlığını artırmıştır.

Sonuç: Kuluçkanın 11-16. günleri arasında sıcağa alıştırma (38.8ºC) civciv kalitesini olumsuz etkilemiştir. Ancak, çıkışta artan civciv uzunluğu ve karaciğer oranı değerleri dikkate alındığında sıcağa alıştırma ile birlikte 16A: 8K aydınlatma civciv kalitesi ve kuluçka sonrası performansın iyileştirilmesinde olumlu bir yaklaşım olabilir.

Anahtar sözcükler: Civciv kalitesi, kuluçka performansı, kuluçkada aydınlatma, sıcağa alıştırma ve yumurta kabuk sıcaklığı

INTRODUCTION

Fast growing broiler chickens are susceptible to high ambient temperatures because the capacity of broilers to lose heat through thermoregulatory pathways have not increased in the same proportions as muscle mass through the long years of selection process (Yahav et al., 2004). Intensive selection for higher growth and muscle accumulation in broiler chickens resulted in increased heat production due to high metabolic rate (Gabriel et al., 1996) and broiler producers faced significant economic losses due to heat stress as a result of poor thermo-tolerance of fast growing broilers (Renaudeau et al., 2012; Lara & Rostagno, 2013).

Different environmental and dietary strategies were suggested to overcome detrimental effects of heat stress on broilers (Yahav, 2009). However, a large accumulation of information during the last decade has put two main approaches forward to solve this problem (Lara & Rostagno, 2013). The first one is genetic selection of broilers for increased heat tolerance (Deeb and Cahaner, 2001, 2002) that requires long-term approaches and increase the production cost. Another effective way to cope with heat stress in poultry is epigenetic "temperature adaptation" mechanisms (Decuypere & Bruggeman, 2007; Yahav, 2009). Because, cyclic high temperatures (38.5-39.5ºC) during incubation may cause changes in gene expressions in favour of a better adaptation to heat stress (Costa et al., 2020). A large number of studies reported that increasing the incubation temperature at critical stages (between ED8 and 18) of the embryonic period (thermal manipulation, TM), improved growth and adaptation of broiler chickens to heat stress at post-hatch (Yalcin et al., 2008; Piestun et al., 2009; Al-Rukibat et al., 2017). A critical period for improved thermotolerance between ED8 and ED18 during the embryogenesis was suggested (Uni & Yahav, 2010). Because functional maturation of the hypothalamus in chicken embryo, which controls thermoregulation and metabolic rate, takes place between 11-16 days of embryogenesis and both hypothalamus-pituary-thyroid and hypothalamus-pituary-adrenal axis are functional between ED16 and ED18.

The positive effects of thermal manipulation during incubation rely on several changes at cellular and molecular levels affecting physiological and metabolic systems of embryo (Loyau et al., 2014; Vinoth et al., 2015). High temperature manipulations during late embryogenesis (ED16-ED18) increased muscle accumulation by increased satellite cell proliferation (Halevy et al., 2006). Recently, 39ºC between E12 and E18 ranging between 9 to 18 h daily improved muscle growth and development upregulated expression of several muscle markers and growth factor genes and these changes were followed by increased body weight at slaughter age (Al-Zghoul et al., 2015).

In a recent study, Narinç et al. (2016) reported that high thermal environment (39.6ºC) between ED10 and ED18 did not affect embryo morphology, chick weight and developmental stability of bilateral traits. However, hatchability and chick quality significantly reduced as compared to the control (37.8ºC) temperature treatment. Although the authors did not measure EST in their study, they concluded that high temperature manipulation during embryogenesis should be short term to avoid adverse effects on hatching performance of broilers.

Another important environmental factor along with temperature in poultry management is light. Light affects many physiological functions that show circadian rhythms in living organisms. Light stimulation during incubation affects embryo development, hatchability, and incubation time (Huth & Archer, 2015; Archer, 2017; Tong et al., 2018). As poultry embryos have photosensitive pineal glands and are significantly affected by light (Zeman et al., 1992). Therefore, the circadian biological rhythms associated with the hormone melatonin, which is secreted from pineal, can be developed by photoperiodic lighting schedules during the embryonic period. A photoperiodic lighting (16h light and 8 h darkness, 16L: 8D) during incubation increased ability of the chicks to cope with the stress at post-incubation environment (Ozkan et al., 2012). Other studies also reported reduced fear and stress in broilers with an incubation lighting program containing at least 12h light (Archer & Mench, 2017), improvements in hatchability, chick quality

(Archer, 2017), increased embryo and post-hatch muscle growth was observed with light stimulation during incubation (Halevy et al., 2006). Recently, it has also been reported that cyclic lighting during incubation, besides the increase in embryo weight, positively affected bone development and it can be used to reduce leg problems in broiler chickens (Van Der Pol et al., 2019).

Recent studies revealed that monitoring embryo temperature through EST rather than set incubator temperature is more effecient and effective to control temperature requirements of the embryo (Lourens et al., 2005; Meijerhof, 2009). Therefore, this study was designed to study the effect of EST which is only 1ºC higher (38.8ºC) from optimum EST (37.8ºC) for a short time (6h per day) between ED11 and ED16 to avoid negative effects of long term high EST on hatchability, chick quality and organ growth. We further hypothesized that photoperiodic lighting (16 h light and 8 h darkness, 16L: 8D) throughout the incubation in combination with cyclic higher EST may have positive effects on above mentioned parameters. The aim of the study is to investigate the effect of cyclic higher EST and a 16L: 8D lighting during the incubation on hatching performance, hatching time, chick quality and organ growth at hatch. The study was composed of two consecutive experiments.

MATERIAL and METHODS

Animal care and use in this experiment was approved by the Local Ethical Committe for Animal Experiments of Ege University (No: 2020-068). This experiment was carried out between September-November 2020 at Animal Research Unit, Department of Animal Science, Ege University, Izmir.

This study was composed of two consecutive experiments using a total of 840 hatching eggs obtained from the same ROSS 308 commercial broiler breeder flock. The age of breeder flock was 36 and 43 weeks old in experiment 1 and experiment 2, respectively.

Incubation environment and egg shell temperature

Experiment 1. Thermal manipulation

A total of 420 eggs were incubated in this trail using 4 incubators of the same brand (VHS, Turkey). The treatment and control groups were replicated in two incubators. Each replicate incubator contained 105 eggs in three egg trays (35 eggs / tray). A standard incubation condition was maintained in both the incubators with EST of 37.8ºC in control group (Control). In the treatment group (Heated), EST was increased to 38.8ºC for 6 hours between 10: 00 and 16: 00 h.every day from day 11 to 16 (ED 11-16). Thus, heated embryos were exposed to 1ºC higher EST than the control group for 6 h/day during the thermal manipulation period. Humidity was set to 60% in all incubators. The temperature and humidity inside the incubators were monitored regularly using data loggers (EBI-2, EBRO Electronic GmbH & Co. KG, Ingolstadt, Germany). EST was measured once in the morning on the 6 marked eggs in each incubator (6x2 = 12/treatment) to make adjustments in incubator settings using an infrared thermometer (Testo 845, Lenzirch Germany). In Heated group, EST was measured twice after incubator temperature was raised. EST reached to 38.8ºC within 20-25 minutes after incubator temperature was raised up and the same duration was necessary for returning to normal EST of 37.8ºC after temperature setting was adjusted.

In both of the Control and Heated treatments, EST was ranged between 37.7 to 37.8 ± 0.1ºC and 37.8 to 37.9 ± 0.1ºC during the first 10 days and on ED17-18, respectively. There was no difference between Control and Heated treatments in these ED. Means for EST's of Control and Heated groups, before and during thermal manipulation, between ED11-16 are presented in Figure 1.

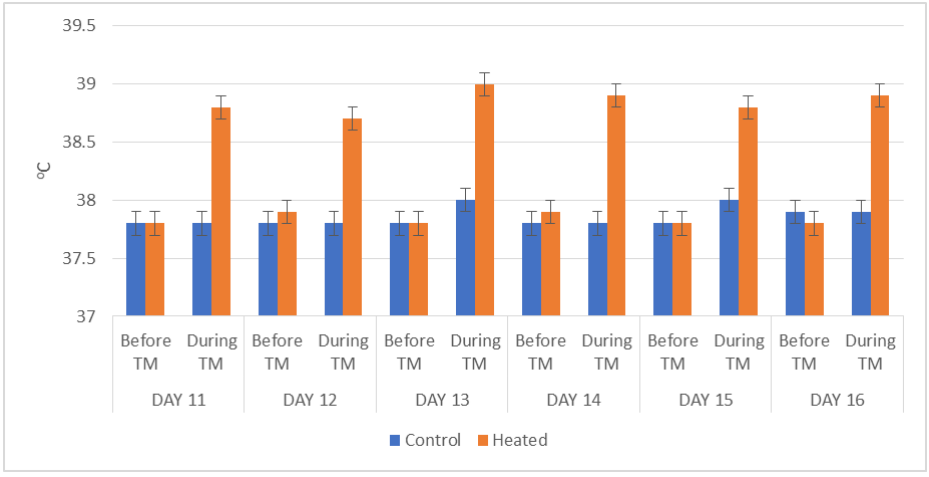

Figure 1. Mean EST before and during thermal manipulation in Control and Heated treatments between ED 11-16.

Şekil 1. *Embryo dönemi 11-16 günler arasında Kontrol ve Sıcağa alıştırma gruplarında ısıtma öncesi ve ısıtma sırasında ortalama yumurta kabuk sıcaklıkları.*

Experiment 2. Photoperiodic lighting and TM

In this trial, all of the four incubators were equipped with cool white LED strips (Samsung 2835, Korea) and 16L: 8D lighting program was applied daily during the whole incubation period (ED0-21). Light was provided between 08: 00 and 24: 00 hours which was controlled by an automatic time clock. The average light intensity was 150 lux at the eggs level. Standard incubation conditions were provided in two incubators i.e. 37.8ºC, with an exception of light provision [Light-Control (LC)]. In the other two incubators, embryos were exposed to increased EST of 38.8ºC for 6 hours every day between ED11-16 [Light-Heated (LH)], following similar experimental procedure as in the first experiment. A total of 420 eggs of the same breed were used in this trial too.

In experiment 2, mean EST of both groups ranged between 37.8 to 37.9 ±0.1ºC during the first 10 days and during the ED17-18 with no significant difference between treatments. Figure 2 presents mean EST of treatment groups which are significantly different ($P<0.05$) from each other during the thermal manipulation period (ED11-16).

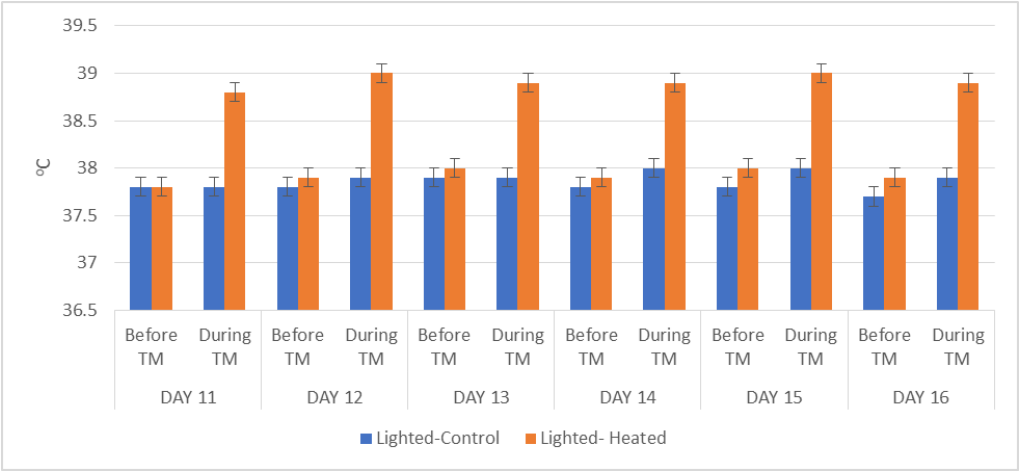

Figure 2. Mean EST before and during thermal manipulation in Lighted-Control and Lighted-Heated treatments between ED 11-16.

Şekil 2. *Embryo dönemi 11-16 günler arasında Aydınlatma-Kontrol sıcaklık ve Aydinlatma-Sıcağa alıştırma grouplarında ısıtma öncesi ve ısıtma sırasında ortalama yumurta kabuk sıcaklıkları.*

Measurements and sample collection in both experiments

Eggs were weighed individually and numbered upon incubation in both trials. Before incubation, 12 random eggs were selected to measure the egg quality traits for each experiment. The only quality parameters differed between two experiments were absolute egg yolk weight and diameter being significantly higher in experiment 2 as compared with the experiment 1. This was in line with the expectation of an increased yolk weight with the increase in age of breeders (Vieira et al., 2005).

Egg weight loss was measured by weighing 6 eggs from each tray in all incubators with a total of 36 eggs per treatment on 18th day of incubation. Eggs were transferred to hatching baskets at the end of ED18. From 464 hours of the incubation period, the number of chicks hatched were recorded at 8-hour intervals. After the hatching was completed all unhatched eggs were broken to determine the early (1-6 days), intermediate (7-14 days), late (15-21 days) mortality (Hamburger & Hamilton, 1992). Embryos that cracked shells but were not able to complete hatching were described as pipping. Hatchability and embryo mortalities were presented as a percentage of fertile eggs set.

At 472 hours of incubation, a total of 16 chicks were randomly selected from each treatment.After the body weight was recorded chicks were killed by neck cut in order to measure organ weights, such as residual egg yolk, liver, heart, breast muscle, spleen and bursa fabricus weights. Relative weights of organs to live body weight were calculated (organ weight/body weight *100). After hatching was completed at 496 h, chicks were removed from the incubators and were weighed (90 chicks/treatment). Thirty chicks per treatment were randomly chosen for chick length measurement and body scoring (with a maximum score of 100) in order to evaluate the chick quality according to the scoring method of Tona et al. (2005).

Statistical Analysis

Data from each experiment, separately, were subjected to ANOVA with fixed treatment effect (Heated and Control in the first experiment; LC and LH in the second experiment) and incubator (replicate) as random effect. The student t-test was used to compare the means for the significant effects. Differences were considered significant at $P < 0.05$. JMP statistics package (SAS, 2002) was used in the statistical analysis of the data.

RESULTS

Experiment 1

Table 1 presents Heated and Control group means for egg weight at the beginning of experiment, moisture loss on ED18, hatchability and embryo mortalities. Egg weights at the beginning of incubation were not different between the treatments. Higher EST did not significantly affect any of the traits measured regarding to hatching performance.

Table 1. Means of treatments for egg weight (g), moisture loss (%) at ED18, hatchability and mortality rates (%)
Çizelge 1. Yumurta ağırlığı (g), ED18'de nem kaybı, Kuluçka randımanı ve ölüm oranları (%) için grupların ortalamaları

		Egg weight (ED0)	Moisture loss	Hatchability	Early mortality	Mid age mortality	Late mortality	Piping	Total mortality
Treatment	Control	59.95	8.30	89.73	2.92	2.21	2.94	2.21	10.27
	Heated	59.51	8.82	94.32	1.56	0.56	1.49	2.07	5.68
	SEM	0.18	0.21	2.45	0.94	0.75	1.14	1.61	2.50
					P-value				
Treatment		0.0834	0.0872	0.1046	0.3295	0.0897	0.3925	0.8938	0.1046

SEM= Standard error of means.

Figure 3 depicts the effect of TM during incubation on hatching time. Hatching rate was significantly higher in heated group at earlier periods of incubation (from 464 to 480 h) (P≤0.05). In heated group, 17.30% of chicks were hatched at 464 h, it was only 1.67% in the control group. Hatchability did not differ between the treatments at 488 h and was about to be completed in both of the groups.

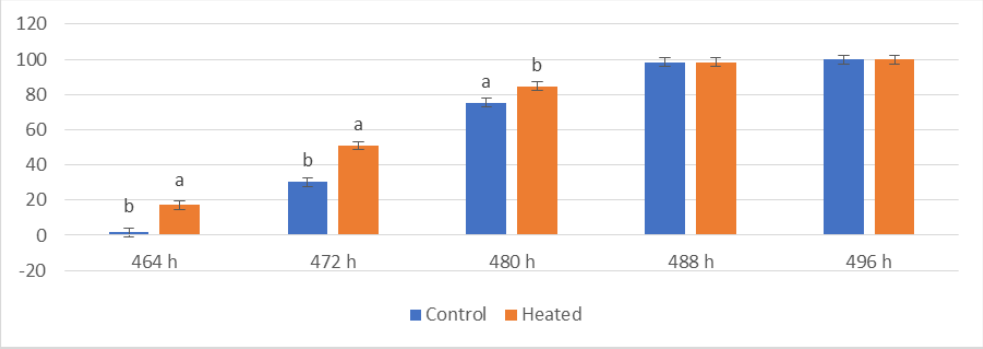

Figure 3. Cumulative hatching rates (%) of Control and Heated groups observed at 8 hours intervals. a,b: means bearing different letters indicate significant difference between treatments at each time point (P<0.05).

Şekil 3. *Kontrol ve Sıcağa alıştırma gruplarının 8 saatlik ara ile gözlenen kümülatif çıkış oranları (%). a, b: farklı harfler taşıyan ortalamalar gruplar arasında önemli bir fark olduğunu gösterir (P <0.05).*

The effect of thermal manipulation during incubation on chick quality was tabulated in Table 2. Weight and chick length from either of the treatments did not differ significantly but chick quality score was found to be significantly lower in the Heated chicks (P≤0.05).

Table 2. Effect of heated incubation on chick quality (length (cm), weight (g) and body score (the highest score = 100)

Çizelge 2. *Kuluçkada sıcağa alıştırmanın civciv kalitesine etkisi. Civciv zunluğu (cm), ağırlık (g) ve vücut skoru (en yüksek skor = 100) üzerindeki etkisi*

Treatment	Chick weight	Chick length	Chick quality score
Control	44.55	18.39	93.60[a]
Heated	43.42	18.21	82.53[b]
SEM	0.46	0.10	1.23
		P-value	
Treatment	0.0931	0.2227	0.0001

SEM= Standard error of means. a,b: means bearing different letters indicate significant difference between treatments (P<0.05).

The effect of thermal manipulation during incubation on organ weight at hatch are given in Table 3. Relative Bursa of fabricus and tibia weights of chicks from Heated group were lower than that of the Control group (P<0.05). However, significantly higher relative weights of heart and sternum were observed in Heated group as compared with the Control (P<0.05). Spleen, residual yolk, liver and breast muscles did not differ with treatments.

Table 3. Effect of heated incubation on relative weights (%) of organs at hatch

Çizelge 3. *Kuluçkada sıcağa alıştırmanın çıkıştaki oransal organ ağırlıkları (%) üzerindeki etkisi*

		Bursa	Spleen	Yolk	Heart	Liver	Breast muscle	Tibia	Sternum
Treatment	Control	0.100[a]	0.03	14.62	0.62[b]	2.00	1.66	0.83[a]	0.48[b]
	Heated	0.081[b]	0.03	14.55	0.68[a]	2.06	1.58	0.71[b]	0.63[a]
	SEM	0.006	0.003	0.44	0.02	0.06	0.04	0.02	0.02
					P-value				
Treatment		0.0341	0.5057	0.9167	0.0156	0.5248	0.1758	0.0004	<.0001

SEM= Standard error of means. a,b: means bearing different letters indicate significant difference between treatments (P<0.05).

Experiment 2

The means of egg weight at the beginning of experiment 2, moisture loss on ED18, hatchability and embryo mortalities from LC and LH groups are given in Table 4. Egg weights of experimental groups were not different at the beginning of the experiment. However the amount of moisture loss in the eggs from LH group was significantly higher (9.82%) than LC (8.27%) (P<0.05). No significant difference between treatments were observed for hatchability and mortality.

Table 4. Means of treatments for egg weight (g), moisture loss, hatchability and mortality rates (%)

Çizelge 4. *Yumurta ağırlığı (g), ED18'de nem kaybı, Kuluçka randımanı ve ölüm oranları (%) için grupların ortalamaları*

		Egg weight (ED0)	Moisture loss	Hatchability	Early mortality	Mid age mortality	Late mortality	Piping	Total mortality
Treatment	Lighted-Control	61.91	8.27[b]	90.52	1.52	4.01	3.18	0.78	9.48
	Lighted-Heated	62.47	9.82[a]	89.76	2.45	3.39	3.43	0.97	10.24
	SEM	0.22	0.35	2.73	1.11	2.32	1.68	0.71	2.82
P-value									
Treatment		0.0728	<.0001	0.8295	0.4812	0.8063	0.8324	0.8559	0.8295

SEM= Standard error of means. a,b: means bearing different letters indicate significant difference between treatments (P<0.05).

Figure 4 represents the mean hatching rates of LC and LH groups by time. No significant difference was observed in either of the treatment groups at any time points.

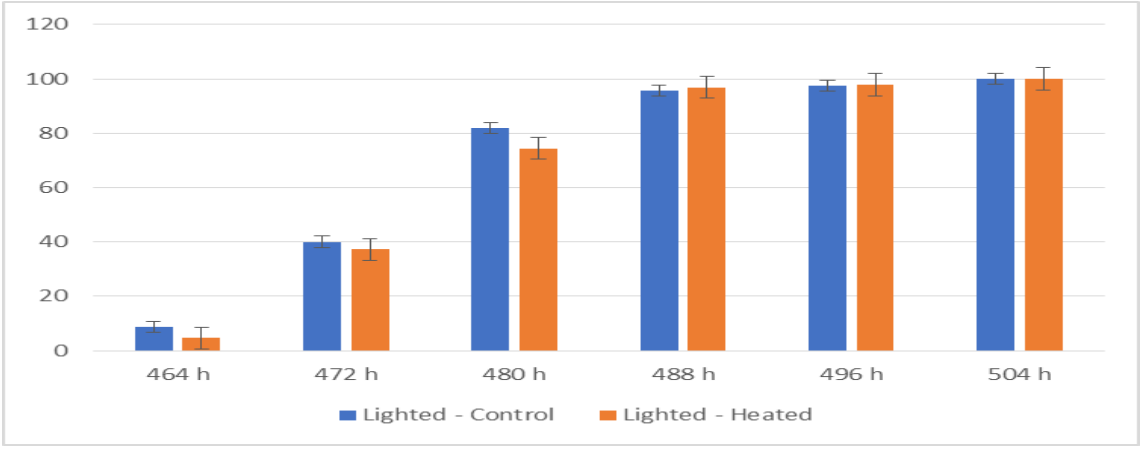

Figure 4. Cumulative hatching rates (%) of Lighted-Control and Lighted-Heated groups observed at 8 hours intervals.

***Şekil* 4.** Aydınlatma-Kontrol ve Aydinlatma-Sıcağa alıştırma gruplarının 8 saatlik ara ile gözlenen kümülatif çıkış oranları (%).

The effect of LC and LH incubation on chick quality are given in Table 5. Weight of chicks at hatch from either of the treatments did not differ significantly but chick length did (P<0.05). Chicks from LH group were significantly longer than those in LC group (P<0.05). However, chick quality score did not differ with the treatments.

Table 5. Effect of Lighted-Control and Lighted-Heated incubation on chick quality (length (cm), weight (g) and body score (the highest score = 100)

Çizelge 5. *Kuluçkada Aydılatma-Kontrol ve Aydinlatma-Sıcağa alıştırmanın civciv kalitesi üzerindeki etkisi . Civciv uzunluğu (cm), ağırlık (g) ve vücut skoru (en yüksek skor = 100)*

Treatment	Chick weight	Chick length	Chick quality score
Lighted-Control	46.18	18.08[b]	96.26
Lighted-Heated	46.45	18.35[a]	97.73
SEM	0.75	0.08	0.60
		P-value	
	0.8035	0.0205	0.0902

SEM= Standard error of means. a,b: means bearing different letters indicate significant difference between treatments (P<0.05).

The effect of LC and LH incubation on organ weights at hatch are presented in Table 6. Relative weights of organs did not differ with the treatments with the only exception of liver weight. LH treatment significantly increased relative weight of liver in day old chicks as compared to the LC (P<0.05).

Table 6. Effect of Lighted-Control and Lighted-Heated incubation on relative weights (%) of organs of chicks at hatch

Çizelge 6. *Kuluçkada Aydınlatma-Kontrol ve Aydinlatma-Sıcağa alıştırmanın çıkıştaki oransal organ ağırlıkları (%)ı üzerindeki etkisi*

		Bursa	Spleen	Yolk	Heart	Liver	Breast muscle	Tibia	Sternum
Treatment	Lighted-Control	0.11	0.04	15.07	0.64	1.83[b]	1.67	0.67	0.63
	Lighted-Heated	0.09	0.04	14.6	0.64	2.10[a]	1.70	0.67	0.68
	SEM	0.01	0.003	0.54	0.02	0.06	0.05	0.02	0.03
					P-value				
Treatment		0.1418	0.5668	0.5379	0.7916	0.0042	0.6193	0.9601	0.2005

SEM= Standard error of means. a,b: means bearing different letters indicate significant difference between treatments (P<0.05).

DISCUSSION

Experiment 1

Incubation conditions are important to obtain desired hatchability and chick quality by adjusting them according to the embryonic requirments (Meijerhof, 2009). TM during incubation is one of the most important factor that affects hatching performance, embryonal stress, and chick quality along with impact on growth and development of the embryo (French, 1994; Christensen et al., 1999). It is important to develop a suitable TM program which would improve thermotolerance and posthatch muscle accumulation without any detrimental effect on hatching performance and hatching time. Thermal manipulation at embryonic age of broiler chickens was identified as a unique management tool that enables broiler chickens to cope with high environmental temperatures (Uni and Yahav, 2010). The present study focused to investigate the effect of a mild cyclic high temperature (EST of 38.8ºC, 1ºC higher than optimum EST of 37.8ºC) and photoperiodic lighting during the incubation on hatching performance, hatching time, chick quality and organ growth.

Moister loss from the eggs during incubation is vital for proper embryo development and successful hatching as explained by Boleli et al. (2016) in their review, which also influence the hatching time and hatching quality by the end of hatch. Moister loss was not significantly affected by thermal manipulation in Experiment 1 which was in agreement with the observations of Aminoroaya et al. (2016) who reported no influence of TM on egg weight loss while exposing eggs to a higher temp of 39ºC for 3 h/d at either ED 12-

14 or 15-17. While Piestun et al. (2011) (eggs exposed to 39.5ºC for 12 h/d, during E7-E16) and Amjadian and Shahir, (2020) (39.5ºC for 3 h/d during ED11 to ED16) showed that the application of heat during incubation significantly reduced egg weight due to the high incubation temperature. Therefore, it could be concluded that TM applied in this study were not high enough to yield a significantly increased moisture loss from the eggs.

Thermal manipulation did not affect the hatchability in the current study with a numerical improvement which is in line with some early studies (Yalcin &Siegel, 2003; Yalcin et al.,2008; Piestun et al., 2008a, b; Amjadian & Shahir, 2020; Saleh et al., 2020). However, some other studies observed significant improvement in hatchability of TM groups (Yahav et al., 2004; Collin et al., 2007; Halle & Tzschentke, 2011; Al-Zghoul et al., 2015). Other studies have reported that exposing eggs to TM during incubation decreased hatchability (Al-Zghoul et al., 2015; Narinç et al., 2016; Al-Zghoul & El-Bahr, 2019) which is in contrast to these results. This could be attributed to the fact that the increased EST used in those studies which was around 40-40.5ºC (Piestun et al., 2013; Al-Zghoul & El-Bahr, 2019) during the TM. However, in this study EST in the TM group was 38.8ºC. Indeed, differences between the experiments can be clarified by the different duration, and timing of temperature used during TM (Costa et al., 2020; Saleh et al., 2020). So, we can conclude that a thermal manipulation scheduled between ED11-16 with an EST of 1ºC higher than optimum (37.8ºC) did not result in any detrimental effect on hatchability, even though hatchability was numerically improved in this study.

Results indicated that TM accelerated the hatching process. The acceleration of the hatching induced by high incubation temperature was previously reported in broilers when TM was carried out for 12 h between E7-E16 (Piestun et al., 2008a). Also, there was no significant difference between treatments in chick weight and length but total chick quality score was significantly reduced in Heated group as compared to that of Control group ($P<0.05$). Another study also observed that the application of TM during incubation decreased chick quality (Sgavioli et al., 2016). The decrease in chick quality might be due to decrease in incubation duration, which results in a shorter time for embryos to use nutrients of the yolk and develop (Molenaar et al., 2010) or due to excessive moisture loss during embryogenesis resulting in shrinkage and weakening of embryos (Sözcü & İpek, 2013). In our study, Heated incubation treatment accelerated hatching time. Although not significantly differed between treatments, a numerical increase in moister loss and slight reduction in BW in Heated group was observed. However, reduced chick quality in Heated incubation treatment might be more related to accelerated hatching time in Heated group. Heated group hatched earlier and stayed longer in the incubator till pull out of chicks when hatching was completed at 496 h. Chick quality significantly differs among day old chicks due to spread of hatch was stated in a study (Tona et al., 2003). The importance of spread of hatching, i.e. early or late hatching time was further reported (Careghi et al., 2005). They observed that increased holding time due to spread of hatch resulted in reduced chick weight and reduced growth in early hatched chicks. Furthermore, early hatched chicks could have benefited more if they had early access to feed and water. Zaboli et al. (2017) reported that a delay in the hatching process with a decreased body weight of chicks obtained from TM of 39.5ºC for 12 h/d from ED 7 to 16 which is in contrary to findings of our experiment. We did not find any significant adverse effects on body weight at hatch by TM but an acceleration in hatching time indicating that 1ºC increase in EST for 6 h/d may be a threshold on hatching performance. However, a decrease in relative weight of bursa in Heated group might be indicative of suppression in immune function. Because, the animal's immune function is influnced by the changes in immune organ measurements. In poultry, bursa of fabricius and spleen are important humoral and peripheral immune organs which plays a vital role in cellular and humoral immunity. However, relative weight of spleen did not differ with the treatments in this study. Our results of reduced bursa weight resembled to those of Liu et al. (2013) who also found that immune organ developmnet was reduced by application of high incubation temperature. In line with our findings another study reported that atrophy and lesions in bursa of day old chicks have been observed in response to "hot and slight hot" thermal stimulation of broiler

embryos between ED14-18 with a TM duration of 3 and 2 h per day (Flores et al., 2016). In the study of Flores et al. (2016) mean EST was measured as 38.8ºC for TM groups as similar to our experimental setting. One of the reason might be that Increased temperature might have activated the body's endocrine system of hypothalamic-pituitary-adrenal (HPA) development resulting in reduced immune function which repressed bursa growth (Gong & Zhong, 2009).

We observed that there was significant increase in relative heart and sternum weights to body weight but a decrease in tibia weight of chicks from Heated incubation. Heart size can be evaluated to determine the heart rate and oxygen metabolism during embryogenesis. A study recorded increase in heart rate and oxygen consumption (Piestun et al., 2009) indicating hyperplasia of the heart which is accordance to our findings. Our results were contrary to (Yalcin et al., 2008; Molenaar et al., 2011; Ipek et al., 2015) who observed significantly lower relative heart weight at hatch and an increased susceptibility to ascites at slaughter age when a high EST of 38.5-40.0ºC were applied between ED10-18 of the incubation or 38.9ºC from day 7 till hatch. Increased heart weight has often shown to be related with higher metabolism which needs more oxygen. Hence increased heart in this experiment indicates that our TM application (EST of 38.8ºC) did not result in any negative effect on heart size and ascites susceptibility as it was reported in earlier TM studies.

Thermal manipulation during Incubation has effect on bone development during embryogenesis and post-hatch (Oviedo-Rondón et al., 2008, 2009; Van Der Pol et al., 2014). In the present study a reduced weight of tibia was observed in chicks exposed to EST of 38.8ºC during incubation which is line with (Yalçin et al., 2007) who found lower tibia weights at hatch for eggs exposed to high (39.6ºC) incubation temperatures between ED10 and ED18. Also, an increased EST (38.6°C) during incubation resulted in reduced tibia, femur, and metatarsus lengths at hatch in comparison to reduced (36.9ºC) and control (37.8ºC) EST's (Van Der Pol et al., 2014) . In another study, 1.0°C higher incubation temperature (38.8ºC vs. 37.8ºC) from E10 onward negatively affected tibia characteristics, including growth plate development, at different sampling days before and at hatch (Oznurlu et al., 2016). There is no evidence of improved sternum weight on TM during incubation in earlier studies but with the increase in temperature during incubation increased satellite cell proliferation (Halevy et al., 2006) can be the reason for improved in sternum weight. Increased sternum weight may indicate stronger sternum which is positive for poultry welfare. However, this finding needs further investigation.

Experiment 2

In the present study, overall chick score was not significantly different but chick length significantly improved in LH group in which eggs were exposed to a cyclic lighting regime along with TM. Improvement in chick length can be considered as improvement in chick quality and these results are in line with other studies which exposed the eggs to light during incubation (Archer and Mench, 2014; Huth and Archer, 2015; Archer, 2016; Archer et al., 2017). Therefore, our observations for increased chick length in the LH treatment, might be due to a positive effect of light in combination with the mild thermal manipulation on the embryos. When eggs were exposed to a photoperiodic lighting, melatonin secretions are stimulated and starts to establish a circadian rhythm in pineal from ED16 (Zeman et al., 1992). Chiandetti et al. (2013) further suggested that photo-sensitive areas in different brain regions other than retina and pineal are being developed as early as three days of embryo development indicating possible epigenetic changes that may occure in the embryos affecting posthatch physiology and behavior of chicks due to lighted incubation (Ozkan et al., 2012; Chiandetti et al., 2013). We may conclude that improved chick quality in LH treatment might be resulted from accelerated bone growth caused by light (Van Der Pol et al., 2019) in combination with heat (Uni and Yahav, 2010) exposure resulting in improved chick length. Results indicate that there is no significant difference between the LC and LH groups in terms of hatching time and hatching performance.

Larger relative weight of liver in day old chicks from LH treatment was in line with the earlier reports. Zhang et al. (2014) found that liver weights were increased when broiler eggs were exposed to green LED light during incubation as compared to dark-incubated chickens. Higher liver weights were also observed in eggs exposed to high temperature (Leksrisompong et al., 2007). As known low liver glycogen content is associated with depressed embryo survival (Christensen et al., 1999) while higher liver weight indicating more liver glycogen accumulation which is an indicator for improved growth at posthatch (Willemsen et al., 2011).

CONCLUSION

Our first hypothesis was that a mild and shorter duration of TM used in this study [providing an EST which is only 1ºC higher (38.8ºC) from optimum EST (37.8ºC), 6 h per day between ED11 and ED16] could avoid negative effects of TM on hatching performance and chick quality. Results from the study did not completely support our first hypothesis. Although, Heated treatment did not impair hatching traits, but it reduced chick quality score at hatch as compared with the Control incubation. Furthermore, atrophy in bursa observed in this group may be associated with reduced immune function. Also, decrease in tibia weight indicates impaired leg development. Despite negative findings with application of TM alone, hyperplasia of heart and increased sternum bone weight give positive indication for application of cyclic higher TM during incubation.

Our second hypothesis was that a photoperiodic lighting throughout the incubation in combination with TM may have positive effects on embryo growth, hatching performance, hatching time and chick quality. The only supporting data for our second hypothesis were an improved chick length and increased liver weight at hatch in LH group as a means of embryo growth and chick quality are in line with the findings of Archer (2017) who reported that lighted incubation could be used as a tool for improving chick quality without negative effects on hatchability. Furthermore, there was no difference in immune organ weights between LC and LH groups which may be indicative to a positive effect of lighted incubation when combined with TM on immune organ development.

As a conclusion, it could be stated that TM alone had negative effects on chick quality. However, a 16L: 8D photoperiodic lighting schedule during the incubation seems to be partly over helmed negative effect of TM on chick quality by improving chick length as a means of chick quality at hatch. Also, increase in liver weight indicates higher liver glycogen production which is an indicator for improved growth posthatch.Considering these findings further research would be useful to describe the effects of the treatments on post hatching growth performance and response of birds to heat challenges during later periods of broiler production.

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