Acute and chronic eggshell temperature manipulations during hatching term influence hatchability, broiler performance, and ascites incidence

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ABSTRACT The aim of the current study was to determine how a control temperature and acute and chronic high eggshell temperatures during the last three days of incubation, can affect hatchability, chick quality, and organ development on day of hatch as well as broiler performance and ascites incidence in later life. The eggshell temperature manipulations were applied during hatching term (days 19 to 21) as follows: control EST (37.3 to 38.0°C), acute high eggshell temperature manipulations (38.4- to 39.0°C for three hours daily) and chronic high eggshell temperature manipulations (38.4 to 39.0°C). The lowest hatchability and the highest cull chick rate were in the chronic high eggshell temperature manipulations group. Lower chick quality parameters correlated with lower chick weights and heavier residual yolk sac weights that were in the chronic high eggshell temperature manipulations group depending on hatch time. The live weights on the 1st day of the growing period were higher in the control and acute high eggshell temperature manipulations groups than the chronic high eggshell temperature manipulations group. At 6 wk of age, live weights of broilers were the highest in the control than in the acute and chronic high eggshell temperature manipulations groups. The total mortality was 2.5, 9.2, and 13.3%, the mortality due to ascites was 2.1, 8.3, and 12.9% in the control, acute ,and chronic high eggshell temperature manipulations groups, respectively. The right ventricular/total ventricular ratios for the control, acute and chronic high eggshell temperature manipulations groups were 0.22, 0.28, and 0.30%, respectively. In conclusion, short-term and long-term higher temperatures during the hatching term affect embryo development, incubation results, broiler performance, and ascites incidence. Although the acute high eggshell temperature manipulations did not affect the chick quality parameters at hatch, it negatively affected incubation results and broiler performance, especially mortality due to ascites.

Key words: chronic, acute high temperature exposure, embryo development, broiler performance, ascites

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INTRODUCTION

Incubation temperature is one of the most critical factors during the incubation period for embryonic development, hatchability, and subsequent broiler performance (Willemsen et al., 2010; Leksrisompong et al., 2007; Molenaar et al., 2011a). As embryos with high growing rates seem to be sensitive to temperature fluctuations (Molenaar et al., 2010), control of the embryo or eggshell temperature is more crucial than controlling incubator temperature. In the first stage of incubation, eggs containing live embryos absorb heat from the surrounding air in the incubator, and the embryo temperature is lower than the incubator temperature (French, 1999; Leksrisompong et al., 2007; Pulikanti et al., 2011). Conversely, during the second stage of the incubation period, especially after embryonic day (**ED**) 9, optimum incubation temperature ranges are difficult to reach due to excessive heat production from the developing embryos (Lourens et al., 2007; Meijerhof, 2009).

Optimum eggshell temperature (**EST**) of 37.8°C eggshell temperature until ED 19 is crucial for obtaining the highest hatchability and chick quality with the highest yolk free body weight and longest chick length at hatch (Lourens et al., 2005, 2007; Molenaar et al., 2011a, b). An increased EST higher than 39.5°C after ED 14 retarded organ growth of embryos (Leksrisompong et al., 2007). Moreover, crop, gizzard, proventriculus, liver, and intestine development are suppressed in embryos exposed to higher incubation temperatures (Maatjens et al., 2014), and the heart is the most consistently affected organ by the higher temperature (Wineland et al., 2000a; Leksrisompong et al., 2007). Certain studies have shown that higher eggshell temperatures (**EST**) (39.5°C) after ED 14 decreased relative heart weights between 17 and 31% in broilers (Wineland et al., 2000a, b; Leksrisompong et al., 2007), and it may contribute to metabolic disorders associated with cardiovascular development, such as ascites (Molenaar et al., 2011a). Although the peak incidence of ascites occurs in the fifth or sixth week of the growing

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period, the etiology of the disease may be initiated much earlier, even during the embryonic stage (Coleman and Coleman, 1991).

During the development of ascites syndrome, hematocrit levels, hemoglobin (Hb) levels, and red blood cell (**RBC**) counts all increase (Yersin et al., 1992). An increase in hematocrit levels results in higher blood viscosity and leads to pulmonary hypertension, right ventricular hypertrophy, oedema, and ascites (Julian, 1993; Wideman 2001). The right venticular/total venticular (**RV/TV**) ratio, hemoglobin level, hematocrit level, and specific clinical chemistry parameters can be used to determine the ascites status of a bird prior to the appearance of gross lesions (Huchzermeyer et al., 1988).

Studies are usually performed to compare the effects of lower or higher incubation temperatures to control temperatures during the first 18 days of incubation on embryonic development and embryonic mortalities, incubation parameters, organ development, bone development, and leg problems. There is a lack of experiments evaluating differences between a short duration of higher incubation temperature and constant higher incubation temperature, especially after ED 19, regarding embryonic development and subsequent broiler performance. Thus, the aim of the current study was to determine the effects of acute and chronic high eggshell temperature manipulations (**ESTM**) during the last three days of incubation on hatchability, chick quality, and organ development on day of hatch as well as broiler performance and ascites incidence in later life.

MATERIALS AND METHODS

The care and use of animals were in accordance with the laws and regulations of Turkey and approved by the Ethical Committee of the Uludağ University (License number 2013-15/01).

A total of 1,800 eggs were obtained from a commercial Ross 308 broiler breeder parent stock at 42 wk of age. The eggs were selected with a range of 55 to 65 g, stored at 18°C and 65% relative humidity (**RH**) for 3 days, and warmed to room temperature (22°C) for 8 h before setting. All eggs were numbered, weighed prior to incubation, and then incubated in the same incubator (1,800 capacity single-stage egg setter, T2400 C, Cimuka Inc., Ankara, Turkey) at full capacity at 37.8°C EST and a relative humidity of 55 to 60% during the first 18 days of incubation. During the first 18 days of incubation, EST was followed by an infrared digital thermometer (Braun, Kronberg, Germany) measurement. On the 18th day of incubation, the eggs were candled, and eggs with viable embryos were randomly divided into three groups and placed into hatching baskets (7 hatching baskets, 80 eggs per basket and 560 eggs per treatment group). Each hatching basket was considered a replicate. The eggs were then transferred to three hatcher cabinets (640 capacity egg hatcher, 8 trays; T640 H, Cimuka Inc., Ankara, Turkey) for the following ESTM: control (36.8 to 37.0°C); acute high ESTM (38.8 to 39.0°C for three hours daily and 36.8 to 37.0°C for the remaining time); and chronic high ESTM (38.8 to 39.0°C). The EST during the manipulations was measured daily by contact at the equator of the egg using an infrared digital thermometer (IRT 4520, Thermoscan, Braun, Germany, Figure 1). During the last three days of incubation, the relative humidity was set at 55 to 60%. The CO_2 concentration was maintained between 0.20 and 0.30%.

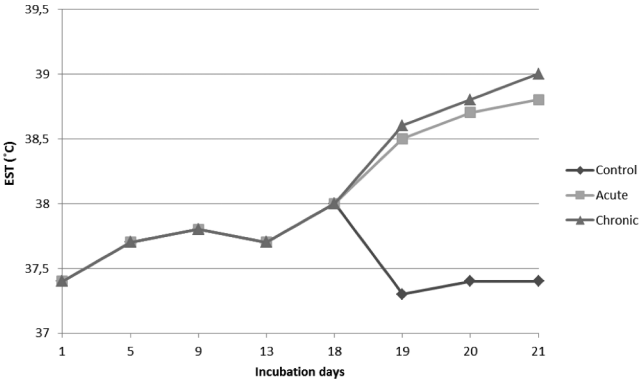

Figure 1. The eggshell temperature profiles in eggshell temperature manipulation groups during incubation (°C).

During the last three days of incubation (from ED 19 to hatch), 5 eggs per incubator tray (n = 35 eggs/treatment group) were randomly sampled to determine embryonic development. On the 21st day of incubation, sample eggs were randomly taken from the unpipped eggs. Sampling was performed regularly at the same time during the experiment. Embryos from each treatment group were killed by cervical dislocation, and the embryo weight and yolk sac weight were measured (Willemsen et al., 2010). Embryos were excised from the extra embryonic membranes and were then carefully separated from the yolk sac. Excessive embryonic fluid was dried off with absorbent paper. Embryos and yolk sacs were weighed to calculate relative embryo and yolk sac weights. Embryo length was measured from the tip of the beak to the tip of the middle toe by placing the chick face down on a flat surface and straightening the right leg (Hill, 2001). Approximately 12 h after hatch (day 1), a total of 35 dry chicks from each treatment were randomly taken and killed by cervical dislocation to determine chick weight, chick length, cloacal temperature, residual yolk sac weight, heart weight, intestine length, intestine weight, and bursa of Fabricius weight. Chick length was measured using the same method to measure embryo length. The cloacal temperatures of the chicks were also measured (to the nearest 0.01°C) using a thermocouple thermometer that was inserted into the cloaca.

The hatched chicks were followed to determine the hatch time for the treatment groups. At hatch, chicks were classified as saleable or cull (Molenaar et al.,

2011a). The hatchability and cull chick rates were expressed as a percentage of fertile eggs (Molenaar et al., 2011a). Unhatched eggs were opened to determine late-term embryonic mortality. Late-term embryonic mortality (**LEM**) values were calculated as follows: LEM = (number of dead embryos at late term/all viable eggs at transfer) × 100. Chick hatching weight was determined by weighing all chicks with using a balance at ±0.1 g precision.

A total of 720 chicks were randomly allocated into treatment groups (control, acute, and chronic high ESTM). The chicks were placed in 18 floor pens with a surface area of 4 m^2 to provide 6 replicate pens and 40 chicks (20 males/20 females) per replicate. Day old chicks were feather sexed. The chicks were weighed using a balance at ±0.1 g precision on the first day of the growing period. Wood shavings laid at a thickness of 8 to 10 cm on the floors of the pens were used as litter material.

The chicks received a standard pelleted broiler starter diet (22.5% CP and ME 12.8 MJ/kg of diet) between days 1 to 14, a grower diet (22.0% CP and ME 13.3 MJ/kg of diet) between days 15 to 28, and a finisher diet (21.0% CP and ME 13.5 MJ/kg of diet) between days 29 to 42. Feed and water were offered ad libitum during the growing period. The chicks were exposed to 23 hours of light and 1 hour of darkness (30 to 40 lux/m^2) until the end of the experiment. Room temperature was 33°C at 1 d of age and decreased to 24°C gradually by 3°C/wk until 21 d of age. After this age, ascites-inducing conditions were provided according to the method of Luger et al. (2001). All groups were reared under regular conditions with gradually reduced room temperature to 14°C and 50 to 60% relative humidity until the end of the experiment (between 22 and 42 days). The live weight values were monitored per pen on a weekly basis until the end of the sixth week. The feed conversion ratio (**FCR**) was calculated on pen basis using the weekly live weight gains and feed consumption values. During the experiment, the feed conversion ratio was corrected for mortality.

The mortality by pen was recorded daily during the trial. All dead birds underwent necropsy to be examined for hydropericardium, right ventricular hypertrophy, and abdominal fluid accumulation from 21 d of age to slaughter age (Shinder et al., 2009). The RV:TV ratio was calculated and broilers with RV/TV ratios above 0.27 were classified as ascitic (Huchzermeyer et al., 1988; Wideman, 2001). Furthermore, broilers with abdominal fluid were also classified as ascitic (Shinder et al., 2009). At slaughter age (on day 42), 30 broilers (15 males and 15 females) were randomly selected from each ESTM group and killed by decapitation. Approximately 1 ml of blood was collected for hematological tests, including the packed cell volume (**PCV**), Hb and RBC assessments using standard methods described by Schalm et al. (1975). The hearts were removed and dissected to obtain heart weights for calculating the RV:TV ratio (Huchzermeyer et al., 1988).

Statistical Analyses

The data were subjected to analysis of variance (SAS Institute, 1989), utilizing ANOVA procedures for balanced data. Analyses for percentage data were conducted after square root of arc sine transformation of the data. Significant differences among treatment means were determined by the Duncan's multiple range test. Body weight and FCR values during the growing period were analyzed using the mixed-effects model (**MIXED**) procedure for repeated measurements, for TM treatments, with the pen as a repeated factor. Measurements taken at slaughter age (i.e., blood and heart parameters) were analyzed using the general linear model (**GLM**) procedure with ESTM treatments. Total mortality and mortality due to ascites were calculated per pen and were analyzed using chi-square tests for the different ESTM treatments. Data are presented as means ± SE. In all cases, a difference was considered significant at $P \leq 0.05$.

RESULTS

The EST profiles during incubation period are shown in Figure 1. The effects of ESTMs on embryonic development parameters are presented in Table 1. On the 19th day of incubation, the lowest yolk sac weight and relative yolk sac weight in addition to the highest embryo weight, relative embryo weight, and embryo length were in the chronic high ESTM group ($P < 0.05$). On the 20th day of incubation, the yolk sac weight, relative yolk sac weight, and embryo length were similar among groups. However, the embryo weight and relative embryo weight were highest in the chronic high ESTM group ($P < 0.05$) with values of 28.1 g and 45.8%, respectively. On the 21st day of incubation, the yolk sac weight and relative yolk sac weight were higher in the control and acute high ESTM groups, but the highest embryo weight, relative embryo weight, and embryo length were in the chronic high ESTM group ($P < 0.01$, Figures 2 and 3).

The effects of ESTMs on incubation results, chick quality parameters, and organ weights are presented in Table 2. The effects of temperature manipulations on late-term embryonic mortalities were significant among the control, acute high ESTM, and chronic high ESTM groups with values of 1.44, 4.59 and 6.05%, respectively ($P < 0.01$). The highest hatchability and lowest cull chick rate were observed in the control group. The hatchability was 97.8, 93.6, and 90.1%, whereas the cull chick rate was 0.36, 1.79, and 3.88%, in control, acute high ESTM, and chronic high ESTM groups, respectively ($P < 0.01$). The effects of different ESTMs on chick hatching weight were significant ($P < 0.01$). The chick hatching weights were 44.5, 44.3, and 42.9 g in the control, acute high ESTM, and chronic high ESTM groups, respectively. The chick weight/initial egg weight was significantly different among groups and was lowest

Table 1. The effects of eggshell temperature manipulations on embryonic development during the hatching term.

		Embryonic Development Parameters					
Incubation Day	Groups	Egg Weight (g)	Yolk Sac Weight (g)	Relative Yolk Sac Weight (%)	Embryo Weight (g)	Relative Embryo Weight (%)	Embryo Length (cm)
19	Control	60.5 ± 0.43	13.4 ± 0.81^{ab}	22.1 ± 1.27^{a}	23.9 ± 2.63^{b}	39.5 ± 4.45^{ab}	15.4 ± 5.42^{b}
	Acute	60.1 ± 0.52	13.8 ± 0.55^{a}	22.9 ± 0.82^{a}	22.9 ± 0.68^{b}	38.1 ± 1.29^{b}	14.9 ± 5.69^{b}
	Chronic	60.9 ± 0.68	12.2 ± 0.67^{b}	20.1 ± 1.21^{b}	26.8 ± 0.96^{a}	44.0 ± 1.62^{a}	16.5 ± 5.65^{a}
	P-Value	*0.137*	*0.012*	*0.006*	*0.08*	*0.016*	*0.002*
20	Control	61.3 ± 0.44	13.1 ± 1.37	21.4 ± 2.21	24.7 ± 1.39^{b}	40.2 ± 2.26^{b}	17.2 ± 9.74
	Acute	61.2 ± 0.55	11.8 ± 1.07	19.3 ± 1.60	26.3 ± 1.65^{ab}	43.1 ± 3.00^{ab}	17.0 ± 7.54
	Chronic	61.3 ± 0.23	11.8 ± 1.68	19.3 ± 2.74	28.1 ± 1.75^{a}	45.8 ± 2.83^{a}	17.8 ± 6.35
	P-Value	*0.894*	*0.275*	*0.264*	*0.019*	*0.022*	*0.272*
21[1]	Control	61.3 ± 0.99	10.5 ± 0.88^{a}	17.2 ± 1.52^{a}	32.6 ± 1.64^{b}	53.2 ± 3.17^{b}	19.1 ± 4.58^{ab}
	Acute	61.1 ± 0.51	9.2 ± 1.17^{a}	15.0 ± 1.86^{a}	30.4 ± 1.88^{b}	49.8 ± 2.81^{b}	18.2 ± 9.56^{b}
	Chronic	60.7 ± 0.47	5.2 ± 2.22^{b}	8.5 ± 3.65^{b}	39.7 ± 3.16^{a}	65.3 ± 5.15^{a}	19.7 ± 3.56^{a}
	P-Value	*0.438*	*0.000*	*0.000*	*0.000*	*0.00*	*0.012*

[a,b,c]Means ±SEM in a row that possess different superscripts differ significantly ($P < 0.01$; $P < 0.05$)

A total of 35 embryos from each group for each sampling day were randomly sampled for measurements.

Relative yolk sac weight (%) = (Yolk sac weight/egg weight) × 100

Relative embryo weight (%) = (Embryo weight/egg weight) × 100

[1]Samples on day 21 were taken from unpipped eggs.

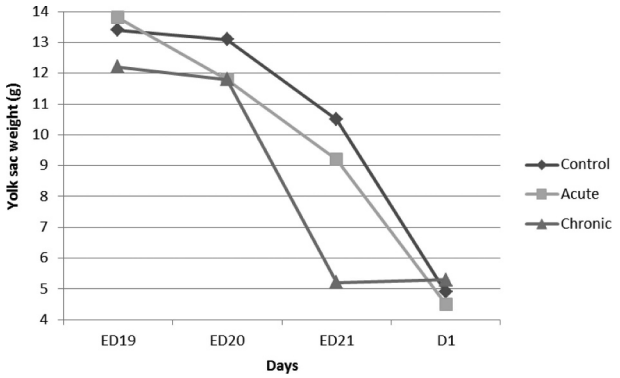

Figure 2. Changes in yolk sac weight during hatching term (ED19-ED21) and day 1 (after hatching) between embryos/chicks in experimental groups.

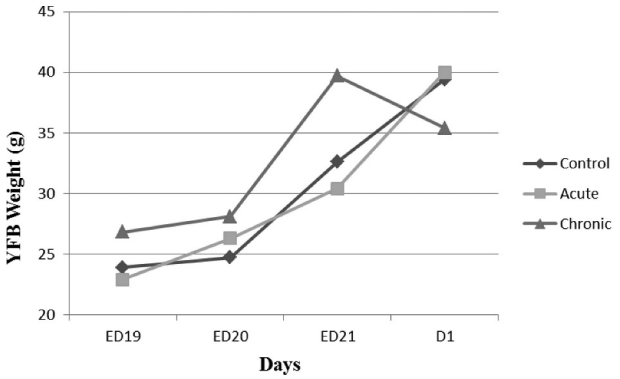

Figure 3. Changes in yolk free body weight (yolk free body, g) during hatching term (ED19-ED21) and day 1 (after hatching) between embryos/chicks in experimental groups.

in the chronic high ESTM group with a value of 66.9% ($P < 0.01$). The hatch time varied at 512, 500, and 495 h in the control, acute high ESTM, and chronic high ESTM groups, respectively. The cloacal temperature was higher in the acute high ESTM and chronic high ESTM groups with values of 40.0 and 40.6°C, respectively, than in the control group with a value of 39.3°C ($P < 0.01$).

Approximately 12 h after hatch, chick quality was measured with chick weight, residual yolk sac weight, and chick length. The chick weights were 44.3, 44.5, and 40.7 g in the control, acute high ESTM, and chronic high ESTM groups, respectively ($P < 0.01$). It was higher in control and acute high ESTM groups than the chronic high ESTM group. Although the residual yolk sac weight was similar among groups, the relative residual yolk sac weight was highest in the chronic high ESTM group with a value of 13.0% ($P < 0.05$). The chick length was similar among groups, with values of 20.0, 19.9, and 20.1 cm in the control, acute high ESTM, and chronic high ESTM groups, respectively ($P > 0.05$). Although the heart, intestine and Bursa Fabricius weights were significantly different among groups ($P < 0.01$), the intestine length was similar among groups ($P > 0.05$). The heart, intestine, and bursa of Fabricius weights were lowest in the chronic high ESTM group ($P < 0.01$). Heart weight was found as 0.54, 0.55, and 0.42 g, intestine weight as 2.1, 2.0, and 1.6 g, with Bursa Fabricius as 0.07, 0.07, and 0.04 g in control, acute high ESTM, and chronic high ESTM groups, respectively.

The effects of ESTMs on the live weight during the growing period, as well as on the cumulative feed consumption and FCR until 42 d of age, are presented in Table 3. The initial body weight on day 1 was higher in the control (44.5 g) and acute high ESTM (44.2 g) groups than in the chronic high ESTM group (42.8 g) ($P < 0.01$). There was a significant difference in the live weights during weeks 1 to 6. The control group was consistently heavier during weeks 1 to 6 than the other groups. At 6 wk of age, the live weight of broilers in the control, acute high ESTM, and chronic high ESTM

Table 2. The effects of eggshell temperature manipulations on incubation results and chick quality parameters and organ weights.

Incubation parameters	Groups			*P*-Value
	Control	Acute	Chronic	
Late Term Embryonic Mortailities (%)	1.44 ± 1.38[b]	4.59 ± 1.48[a]	6.05 ± 3.40[a]	*0.001*
Hatchability (%)	97.81 ± 1.89[a]	93.62 ± 1.97[b]	90.07 ± 4.64[c]	*0.001*
Cull Chick Rate (%)	0.36 ± 0.75[b]	1.79 ± 1.19[a]	3.88 ± 3.12[a]	*0.002*
Chick Hatching Weight (g)	44.5 ± 1.22[a]	44.3 ± 1.08[a]	42.9 ± 1.36[b]	*0.001*
Chick Weight/Initial Egg Weight (%)	72.2 ± 4.84[a]	72.9 ± 4.04[a]	66.9 ± 2.78[b]	*0.007*
Hatch Time (hour)	512	500	495	–
Cloacal Temperature (°C)	39.3 ± 0.49[b]	40.0 ± 0.24[a]	40.6 ± 0.40[a]	*0.001*
Chick Quality Parameters[1] (12 Hours Later After Hatching)				
Chick Weight (g)	44.3 ± 2.91[a]	44.5 ± 2.60[a]	40.7 ± 1.58[b]	*0.004*
Residual Yolk Sac Weight (g)	4.9 ± 0.94	4.5 ± 1.16	5.3 ± 1.40	*0.344*
Relative Residual Yolk Sac Weight (%)	11.1 ± 1.78[ab]	10.11 ± 1.79[b]	13.0 ± 2.99[a]	*0.045*
Chick Length (cm)	20.0 ± 0.60	19.9 ± 0.31	20.1 ± 0.71	*0.651*
Organ Weights[2]				
Heart (g)	0.54 ± 0.05[a]	0.49 ± 0.04[b]	0.42 ± 0.05[c]	*0.001*
Intestine (g)	2.1 ± 0.18[a]	2.0 ± 0.17[a]	1.6 ± 0.20[b]	*0.001*
Intestine Length (cm)	42.3 ± 2.92	39.1 ± 2.90	40.9 ± 4.53	*0.150*
Bursa Fabricius (g)	0.07 ± 0.01[a]	0.07 ± 0.02[a]	0.04 ± 0.01[b]	*0.001*

[a,b,c]Means ±SEM in a row that possess different superscripts differ significantly ($P < 0.01$; $P < 0.05$)

For chick hatch weight, all chicks were weighed individually and, for cloacal temperature, a total of 30 chicks from each group were randomly sampled.

Late term embryonic mortalities (LEM) were calculated: (number of dead embryos at late term/all viable egg incubated) × 100

Chick weight/initial egg weight (%) = (Chick weight/egg weight) × 100

[1,2]A total of 35 chicks from each group were randomly sampled for chick development parameters and organ weights after 12 hours of completing the hatching process.

Relative residual yolk sac weight (%) = (Residual yolk sac weight/chick weight) × 100

Table 3. The effects of eggshell temperature manipulations during incubation on live weight (g) during the growing period as well as cumulative feed consumption (g) and feed conversion ratio (FCR) until 42 d of age after cold challenge.

Parameters	Days	Groups			*P*-Value
		Control	Acute	Chronic	
Live Weight (g)	1	44.5 ± 1.20[a]	44.2 ± 1.12[a]	42.8 ± 1.19[b]	*<0.01*
	7	202.9 ± 7.31[a]	191.9 ± 9.49[b]	174.6 ± 8.82[c]	*<0.01*
	14	512.1 ± 54.58[a]	476.6 ± 26.96[b]	464.0 ± 20.0[c]	*<0.01*
	21*	1053.1 ± 52.7[a]	1021.4 ± 69.9[b]	978.2 ± 59.5[c]	*<0.01*
	28	1628.7 ± 95.5[a]	1590.2 ± 101.6[b]	1597.2 ± 89.8[b]	*<0.01*
	35	2110.6 ± 128.6[a]	2025.2 ± 106.1[b]	2079.9 ± 114.9[b]	*<0.01*
	42	2709.8 ± 132.8[a]	2440.7 ± 122.6[b]	2523.6 ± 112.8[b]	*<0.01*
Cumulative Feed Consumption (g)	1-42	4850.8 ± 109.6[b]	5010.4 ± 161.7[b]	5375.2 ± 329.5[a]	*0.003*
FCR	1-42	1.78 ± 0.04[b]	2.05 ± 0.07[a]	2.13 ± 0.14[a]	*<0.01*

[a,b,c]Means ±SEM in a row that possess different superscripts differ significantly ($P < 0.01$; $P < 0.05$)

*All birds were exposed to cold challenge after d 21 in the same poultry house

groups was 2,709.8, 2,440.7, and 2,523.6 g respectively ($P < 0.01$). Until 42 d of age, the cumulative feed consumption was higher in the chronic high ESTM group (5,375.2 g) than in the control (4,850.8 g) and acute high ESTM (5,010.4 g) groups ($P < 0.01$). During the 6-week growing period, the acute high ESTM (2.05) and chronic high ESTM (2.13) groups had a significantly higher FCR relative to the control group (1.78) ($P < 0.01$).

The effects of the ESTMs on the total mortality and mortality after cold challenge at day 21 due to ascites and the blood and heart parameters of the groups at slaughter age are presented in Table 4. The total mortality was significantly different by groups ($P < 0.01$; 2.5, 9.2, and 13.3% in the control, acute high ESTM, and chronic high ESTM groups, respectively). Similarly, the mortality due to ascites was significantly higher in the chronic high ESTM group (12.9%) than in the acute high ESTM (8.3%) and control (2.1%) groups ($P < 0.01$). The PCV and RBC were highest in the chronic high ESTM group with values of 32.6%, and 2.3 M/mm^3, respectively ($P < 0.01$). The Hb was higher

Table 4. The effects of eggshell temperature manipulations on total mortality (%) and mortality due to ascites (%) during the growing period after cold challenge and the blood and heart parameters at slaughter age.

	Groups				
1-42 d Mortality	Control	Acute	Chronic	Chi-Square	*P*-Value
Total Mortality (%)	2.5 (6/240)	9.2 (22/240)	13.3 (32/240)	17.764	*0.001*
Mortality Due to Ascites (%)	2.1 (5/240)	8.3 (20/240)	12.9 (31/240)	19.789	*0.001*
Blood Parameters (at Slaughter Age)					
PCV, %	27.8 ± 1.5^{c}	31.2 ± 1.3^{b}	32.6 ± 1.4^{a}	-	*0.001*
Hb, g/dl	7.8 ± 0.3^{b}	8.6 ± 0.4^{a}	8.8 ± 0.3^{a}	-	*0.001*
RBC M/mm^3	1.8 ± 0.2^{b}	$2.1 \pm 0.\ 2^{b}$	2.3 ± 0.2^{a}	-	*0.001*
Heart Parameters					
Right Ventricle (RV) (g)	2.38 ± 0.57^{b}	2.76 ± 0.63^{ab}	2.96 ± 0.59^{a}	-	*0.012*
Total Ventricle (TV) (g)	10.60 ± 1.47	9.88 ± 1.58	9.90 ± 1.38	-	*0.241*
RV:TV ratio	0.22 ± 0.04^{b}	0.28 ± 0.05^{a}	0.30 ± 0.05^{a}	-	*0.001*

Numbers in the parentheses are no. of dead birds/total no. of birds

[a,b,c]Means ±SEM in arow that possess different superscripts differ significantly ($P < 0.01$; $P < 0.05$)

For blood and heart parameters measurements, a total of 30 broilers from each group were randomly sampled.

in the acute high ESTM (8.6 g/dl) and chronic high ESTM (8.8 g/dl) groups than in the control group (7.8 g/dl) ($P < 0.01$). Although the RV was significantly different among groups, the TV was similar among groups. The RV was higher in the chronic high ESTM group (2.96 g) than in the control (2.38 g) and acute high ESTM (2.76 g) groups. The RV:TV ratio was 0.22, 0.28, and 0.30% in the control, acute high ESTM, and chronic high ESTM groups, respectively ($P < 0.01$).

DISCUSSION

The aim of the experiment was to determine the effects of acute and chronic high ESTMs during the hatching term on hatchability, chick quality, and organ development on hatching day as well as broiler performance and ascites incidence in later life. The results suggested that long-term high temperature after ED 19 affected these parameters in different patterns compared to control and short-term high temperature.

In commercial conditions, breeder age, egg size, incubator types, and problems with machine maintenance, such as in cooling, may have airflow patterns that result in overheating embryos for brief extended periods (Hulet et al., 2007). Accordingly, a 2 to 4°C difference has been reported between the highest and lowest eggshell temperatures within large-scale incubators on ED 17 of incubation (Hulet et al., 2007; Lourens et al., 2011). So, maintaining the optimum EST during incubation is more important than the machine temperatures (Meijerhof and Van Beek, 1993; Hulet et al., 2007).

In this study, on the 21st day of incubation, the highest embryo weight and relative embryo weight with the lowest yolk sac weight and relative yolk sac weight were observed in embryos from chronic high ESTM group. These results are supported with other studies that have shown that EST influenced yolk sac absorption and embryo development (Lourens et al., 2005, 2007; Molenaar et al., 2011a; Maatjens et al., 2014). Embryos are poikilothermic and have less ability to regulate their body temperature by increasing or decreasing their heat production during incubation (Romjin and Lokhorst, 1955), and therefore, embryonic metabolic rate is largely affected by temperature (Maatjens et al., 2014). It was reported that a high EST of 38.9°C increased metabolic rate and embryonic heat production (Lourens et al., 2007) and that optimum development and highest hatchability were found at EST 37.8°C (Lourens et al., 2005).

An incubation temperature above 39.5°C has negatively affected organ development (Leksrisompong et al., 2007) and chick quality at hatch (Ipek et al., 2014). The quality of a day old chick has been demonstrated to be important for a good start for the chick's life and for subsequent broiler performance (Meijerhof, 2009). Before hatching, uptake of the yolk sac into the abdomen of the embryo provides nutrients for the chick during the first few days of life (Meijerhof, 2009). Approximately 12 h after hatch, the higher chick weights and yolk free chick weights were in the control and acute high ESTM groups, and the lower chick weight was in the chronic high ESTM group. The results of this study agreed with those of previous studies (Leksrisompong et al., 2007; Willemsen et al., 2010; Molenaar et al., 2011a) that concluded that higher temperatures during the late term of incubation negatively affected embryo and chick development. While embryos exposed to higher EST use the yolk sac for maintaining energy for the hatching process, embryos exposed to control EST use the yolk for development of muscle and organs (Willemsen et al., 2010). Molenaar et al. (2011b) applied a normal (37.8°C) and high (38.9°C) EST from ED 7 to ED 19, and they reported a higher chick weight in the normal incubation temperature group.

In contrast to our findings, these authors reported higher residual yolk sac weight in heavier chicks in the normal incubation temperature group. In our study, the similarity in chick weight observed among chicks between acute and chronic ESTM was remarkable. Another study utilizing different elevated temperatures during the hatching period found that chick weight is higher in the normal temperature group than in the high temperature group (Leksrisompong et al., 2007). Similarly, Joseph et al. (2006) found that higher hatch temperature results in a reduction in chick weight at hatch compared to the control temperature because of earlier hatch time.

At higher ESTs above approximately 39°C, the heart is the most affected organ (Wineland et al., 2000a; Lourens et al., 2007; Maatjens et al., 2014). Wineland et al. (2000a) also found that heart weight is reduced at high setter and hatcher temperatures. A study applying different elevated ESTs ranging from 39.7 to 39.9°C during the hatching period reported that the heart was the only organ that showed a significant difference of up to 29% at hatching (Leksrisompong et al., 2007). Similarly, Molenaar et al. (2007) also reported a 26% reduction in the heart weight at hatch in the high incubation temperature group compared to the normal incubation temperature group. Similarly, we found a 9 and 22% reduction in the heart weight of chicks in the acute and chronic high ESTM groups, respectively, compared to the control group. Thus, the reduced heart weights at hatch increase the susceptibility and the incidence of metabolic disorders related to cardiovascular development later in life, such as ascites (Leksrisompong et al., 2007). Furthermore, it has been reported that other organs such as the liver, gizzard, and small intestine, are negatively affected as well by higher incubation temperature (Leksrisompong et al., 2007; Maatjens et al., 2014). In our study, the intestine and bursa of Fabricius were significantly smaller in chicks in the chronic high ESTM group than in chicks in the control and acute high ESTM groups.

During the incubation period, higher incubation temperatures result in lower hatchability, higher cull chick rate, and shorter hatch time (French, 2000; Lourens et al., 2005). Some studies have shown that a high incubation temperature in the second half of incubation can increase embryonic mortality in the last week of incubation (Lourens et al., 2005; Willemsen et al., 2010; Molenaar et al., 2011a). In contrast to our findings, several researchers have concluded that embryonic mortalities are not influenced by incubation temperature (Yalcın et al., 2010; Shim and Pesti, 2011). In our study, lower chick quality parameters correlated with lower chick weight, and a heavier residual yolk sac weight was found in the chronic high ESTM groups. Additionally, higher temperature during the hatching period resulted in a shorter hatch time in the chronic high ESTM group compared to the control and acute high ESTM groups. Similarly, Molenaar et al. (2011b) reported that the incubation period was shorter in high EST 38.9 °C (479 h) than 37.8 °C (487 h). The reduction in incubation period was explained by two possible reasons of less development and nutrient utilization at high EST in their study (Molenaar et al., 2011b).

The effects of incubation temperature fluctuations can impact the economic returns of the poultry industry by affecting posthatch broiler performance and processing yields (Lourens and van Middelkoop, 2000). During the growing period, the effect of ESTMs on live weight was observed where broilers in the control group weighed heavier than the others. Similarly, Hulet et al. (2007) also reported that changes in the incubation temperature during the hatching period affected chick initial body weight with weights of 41.1, 42.2, and 43.1 g in the 37.5, 38.6, and 39.7°C incubation temperature groups, respectively. This variability in the chick initial body weight also reflected in the final body weight at 44 d of age with weights of 2,213.8, 2,263.3, and 2,165.7 g in the 37.5, 38.6, and 39.7°C groups, respectively.

The other broiler performance parameters include cumulative feed consumption, feed conversion ratio (**FCR**), and total mortality. The FCR was highest in the chronic high ESTM group, but it was similar to the FCR of the acute high ESTM group. It may be related to intestinal development at the hatch (Molenaar et al., 2011a). Palo et al. (1995) reported that the development of the gastrointestinal tract has a major role in chick growth during the posthatch early growing period. In the study, the weight of the intestine was lower in chicks from chronic high ESTM group than in others.

Ascites is a major cause of mortality in modern broiler production worldwide (Wideman and French, 2000). During the grow out period, a low temperature with a fast growth rate is one of the main triggers for inducing ascites due to increased metabolic rate and O_2 demand (Scheele et al., 1991; Wideman, 2001). Therefore, ascites-inducing conditions were provided in the study. The total mortality and mortality due to ascites were higher in the acute and chronic high ESTM groups, which clearly showed that the higher temperatures during embryonic development induced ascites. The higher mortality due to ascites in the acute high ESTM and chronic high ESTM groups was supported with the blood and heart parameter results at slaughter age. A high incubation temperature had a negative effect on the pulmonary vascular capacity, and this increase in oxygen metabolic demand lead to the development of ascites (Lubritz and McPherson, 1994). The results of the present study support these findings. In this study, mortality due to ascites in the acute and chronic high ESTM groups was ensured with higher values of PCV, Hb, and RBC. Furthermore, a RV:TV ratio greater than 27% is an indicator for ascites (Wideman, 2001). In our study, the right ventricle weight and the RV:TV ratio was higher in the acute high ESTM and chronic high ESTM groups than in the control group. The RV:TV ratio was 28% and 30% in the

acute high ESTM and chronic high ESTM groups, respectively. Molenaar et al. (2011a) also found that the ratio between the right and total ventricle mass was 1.1% higher in the high EST (38.9°C, from ED 7 to 21) compared to the normal EST (37.8 °C) group at slaughter age.

In conclusion, this study clearly showed that both acute and chronic higher temperatures during the hatching period affected chick quality, incubation results, and subsequent broiler performance. However chronic high ESTM after ED 19, affected these parameters in a different pattern compared to control and acute high ESTM. Acute high ESTM did not affect the chick quality parameters at hatch, but it negatively affected incubation results and broiler performance, especially mortality due to ascites after cold challenge.

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