MANAGEMENT AND PRODUCTION

The effects of eggshell temperature fluctuations during incubation on welfare status and gait score of broilers

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ABSTRACT The aim of the current study was to determine the effects of different eggshell temperatures (EST); low (33.3 to 36.7° C), control (37.8 to 38.2° C), and high (38.9 to 40.0° C) during 10 to 18 days of incubation on welfare status including foot pad dermatitis (FPD), hock dermatitis (HD) and feathering status, and gait score in broilers. Score 2, 4, and 5 of FPD were found to be similar among the treatment groups, whereas a score of 3 was found to be higher in the control and high EST groups (27.7% and 29.2%) compred to the low EST group (16.9%). The eggshell temperature fluctuations were significantly affected the incidence of HD, whereas broiler sex did not. All of the broilers in the high EST group had HD with various scores, while a percentage of 21.1% and 6.9% of broilers had the score 1 of HD in the low and control EST groups, respectively. Feathering status showed a difference between body parts including wing, neck, back, and vent and also a general mean score of broilers from low EST treatment had the highest score for feathering. A higher incidence of gait score was observed in broilers from the control EST treatment than low and high EST groups. This can be attributed to a higher live weight of broilers from the control EST group. On the other hand, the incidence of a gait score of 3 and 4 was found for broilers from control and high EST treatment groups. Male and female broilers from the high EST group had the higher gait score. In conclusion, gait score and welfare status of broilers were affected by fluctuations in EST between 10 and 18 days of incubation.

Key words: gait score, broiler welfare, dermatitis, feathering, eggshell temperature

2016 Poultry Science 95:1296–1303 http://dx.doi.org/10.3382/ps/pew056

INTRODUCTION

Modern breeders of broilers have been selected for higher growth rate, efficiency of feed conversion rate, and also higher breast meat yield (Bessei, 2006). This genetic selection has precipitated some health and thereby welfare problems in broilers including difficulty in walking (Kestin et al., 1992; Sanotra et al., 2001) and lameness (Venalainen et al., 2006). Foot pad dermatitis (**FPD**), hock dermatitis (**HD**), and feathering affect negatively broiler welfare (Breuer et al., 2006). FPD and HD are a kind of contact dermatitis which affects the plantar region of the feet and hock joint (Ekstrand et al., 1997; Mayne, 2005). All of these problems cause welfare problems in terms of pain, discomfort, and inhibition of physical activities (Danbury et al., 2000) and also reduction in productivity and profitability (Breuer et al., 2006).

Gait scoring was developed to measure walking ability in broilers (Kestin et al., 1992). According to this method, broilers are categorised into 6 classes with

Received August 17, 2015.

scores between 0 (healthy broiler with normal, dextrous, and agile walking) and 5 (incapable of walking) (Garner et al., 2002). It is known that some factors such as genetic line, age, sex, and live weight affect the incidence of leg problems, especially lameness and poorer walking ability (Kestin et al., 1999; Sørensen et al., 2000). Thus management factors during the rearing period have great importance in minimizing these problems. Recently however, the incubation period has been considered in respect of possible effects on lameness, FPD, HD, and feathering status of broilers (Oviedo-Rondon et al., 2009a, 2009b; Da Costa et al., 2014; Scott et al., 2015)

Incubation conditions, especially eggshell temperature, affect embryo development and subsequently posthatch broiler performance (Molenaar et al., 2011; Ipek et al., 2014). Deviations from optimum incubation temperatures may affect embryo size, organ and skeletal growth, and hatching success (Yalçin and Siegel, 2003; Tazawa et al., 2004). Wineland et al. (2000a, 2000b) demonstrated that differences in embryo temperature in the setter and hatcher can result in a difference in the development of the whole chicken and specific organs. For example, bone development starts at the embryonic stage (Ballock and O'Keefe, 2003). It could thus be concluded that bone problems could be based on

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Accepted January 21, 2016.

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incubation conditions (Hammond et al., 2007; Yalçin et al., 2007).

Incubation conditions also could be a predisposing factor for FPD and HD in broilers (Da Costa et al., 2014). Epidermal and dermal interaction during embryo development affects the modulation of skin morphology (Sawyer et al., 1984). Besides, dermatitis causes alterations in the skin such as hyperkeratosis (Shepherd and Fairchild, 2010). Similarly, formation of feathers begins at the embryonic stage by interaction of the dermis and epidermis (Scott et al., 2015). Recently, Dahlke et al. (2008) reported that broiler embryos exposed to higher incubation temperatures had a higher density of feather follicles in the dorsal tract, but found any changes in the femoral region. So that, it could be concluded that temperature fluctuations affect follicular development with respect to body region.

Lately, broiler welfare has gained great importance in addition to performance and productivity. This study aims to determine the effects of different eggshell temperatures (low, control, and high) during 10 to 18 days of incubation on serious welfare problems including incidence and severity of FPD and HD, feathering status, and also gait score.

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 Uludag University (License number 2012-01/02).

Hatching Eggs and Incubation

In the study, a total of 1,800 eggs obtained from a commercial Cobb 500 broiler breeder parent stock (35 wk of age) were stored at 16° C and 65% RH for 2 days, and warmed to room temperature $(22^{\circ}C)$ for 8 h before the setting process. All eggs were weighed (55 to 60 g), and then incubated in the same incubator (1,800 capacity egg setter, T2400 C, Cimuka Inc., Ankara, Turkey) at 37.5° C and a RH of 55 to 60% during the first 10 d of incubation. After d 10 of incubation, the eggs were randomly classified into 3 treatment groups and incubated in fully automated ventilated, programmable incubators at full capacity (600 capacity egg setter, 6 trays; T640 I, Cimuka Inc.). Between days 10 and 18 of incubation, the eggshell temperature (EST) was maintained as low EST; control EST, and high EST within the ranges of 33.3 to $36.7^{\circ}C$ (91.9 to $98.0^{\circ}F$), 37.8 to $38.2^{\circ}C$ (100.0 to 100.8°F), 38.9 to 40.0°C (102.0 to 104.0° F), respectively. In this study, the negative effects of excessively high temperatures on incubation results were considered as important, therefore the high EST treatment was applied in a narrower temperature range.

The EST was measured daily by using an infrared digital thermometer (IRT 4520, Thermoscan, Braun, Germany) with contact at the equator of the egg (10

eggs per each tray) from embryonic d 10 to 18. The incubator temperatures were ignored, and the eggshell temperature was considered. The incubator temperatures were programmed daily based on the eggshell temperature. The infrared thermometer was allowed to equilibrate on the floor of an incubator for 10 min before each use. The EST was measured after opening the incubator and then the incubator door was closed.

Hatching Process and Broiler Growing

On 18th day of incubation, the eggs were transferred to the hatcher (1,800 capacity egg hatcher T2400 C, Cimuka Inc.). The hatcher was maintained at 36.2° C and 70% RH during hatching.

After completing hatching, at feather dryness, the chicks (n = 720) were randomly allocated into 3 treatment groups (low, control, and high EST). The chicks were placed in 18 floor pens with a surface area of 2.0 \times 2.0 m² to provide 6 replicate pens and 40 chicks (20 male/20 female) per replicate, and weighed using a balance at \pm 0.1 g precision at the beginning of the trial. Wood shavings were used as litter material, which was laid at a thickness of 7 to 8 cm on the floors of the pens.

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) was fed between days 15 to 28. The finisher diet (21.0% CP and ME 13.5 MJ/kg of diet) was fed between days 29 to 42. Feed and water were offered ad libitum throughout the experiment. The chicks were exposed to 24 hours of light during the first week and 23 hours light and 1 hour of darkness until end of the experiment. Room temperature was 31°C at 1 d of age and decreased gradually by 3°C/wk until it reached 20°C. Room temperature was then maintained at 20°C and 50 to 60% RH until the end of the experiment. The live weight gain values and feed consumption were monitored on a weekly pen basis until the end of the sixth week; feed conversion ratios were calculated using the weekly live weight gains and feed consumption values. The mortality on a per pen basis was recorded daily during the trial.

Litter Characteristics

Samples of the litter were taken on day 42 according to the procedure indicated by Tasistro et al. (2004). Samples of litter were analysed for pH and dry matter. For analysis, samples were collected from three points around the feeders and drinkers and from the central space between feeding and drinking lines.

Foot Pad and Hock Dermatitis Scoring

To evaluate the incidence and severity of FPD and HD, all broilers in each treatment group were scored from four locations (feeders, drinkers, wall edge, and

 Table 1. Scoring of FPD and HD.

Score	Mean
1	No lesions - no evidence for dermatitis
2	Slight occurance of lesions - slight discolouration and hyperkeratosis on a very small area
3	Mild occurance of lesions - discolouration and dermatitis on superficies of foot pad or hock
4	Severe lesions - ulceration and scabs on foot pad or hock
5	Very severe lesions - deep dermatitis with ulceration, scabs, and haemorrhages

Source: Anonymous, 2009.

Table 2. Scoring of walking ability.

0	Normal, agile and well-balanced
1	Slight abnormality and uneven walking but difficult to define
2	Uneven walking with shortened steps, failure in balance, taking support from the wings, definite and identifiable
	abnormality
3	Obvious abnormality, affects ability to move, not standing for more than 15 seconds, after walking lying down
4	Severe abnormality, unwilling to walk, using wings as crutches, only takes a few steps
5	Incapable of walking

Source: Anonymous, 2009.

resting area) at 42 d of age. Scoring was applied with a 1 to 5 rating system according to the severity of lesions. Score "1" means that there is no evidence of FPD or HD, scores "2" and "3" mean that there is minimal evidence of lesions, score "4" means that there is visible evidence of FPD or HD, and score "5" means that there are severe lesions on the foot pad or hock (Anonymous, 2009). After scoring, the scores were calculated as a percentage for the group. The scoring system for FPD and HD is given on Table 1.

Feathering Conditions

To evaluate the feathering status, all broilers in each treatment group were scored at 42 d of age. A scoring was applied with a 1 to 3 rating system by assessing five parts of the body including breast, wing, neck, back, and vent. A score "1" means that the broiler is almost completely covered with feathers, score "2" means that there are large naked areas, and a score "3" means that there are no, or only very small, areas covered with feathers (Tauson et al., 1984). After scoring, the scores were calculated as percentage per group.

Gait Score

At 42 d of age, each broiler was individually monitored for walking ability and evaluated using a gait score ranging from 0 to 5 (Anonymous, 2009). During evaluation, the evaluator sat on the floor at eye level viewing the back of the broiler's legs while it walked for 15 s (Talaty et al., 2010). After scoring, the scores were calculated as a percentage for each group. The assessment system for gait score is given in Table 2.

Statistical Analyses

The data were subjected to analysis of variance (SAS Institute, 1989), utilizing ANOVA procedures for balanced data. In the study, 6 replicate pens (40 chicks per pen) was provided for each treatment group and pens were the experimental unit. Analyses of the percentage data were conducted using a square root of arc sine transformation of the data. Growth performance and welfare parameters were analyzed using the general linear model procedure. The foot pad dermatitis, hock dermatitis, feathering, and gait scores at 41 d of age were analyzed as a 3×2 factorial design that included eggshell temperatures (low, control, high) and broiler sex (male and female). Mean score values of each groups (EST \times sex) were used for statistical analysis. All significant differences were determined by the Duncan's multiple range test. In all cases, a difference was considered significant at P < 0.05.

RESULTS

Broiler Performance

The effects of the EST treatments on the live weight and feed conversion rate were found significantly different at the end of the experiment (on day 42). The initial BWs on day 1 were similar in the low EST (39.61 g) and high EST groups (41.04 g), whereas the control EST group (42.38 g) was heavier (P = 0.024). On day 42, the BW of broilers in the low, control, and high EST groups were determined as 2,172.6; 2,543.9; and 2,282.6 g respectively and the low EST group was found to be lighter (P = 0.001). During the six-week life span, the low (1.86) and high (1.83) EST groups had a significantly worse feed efficiency relative to the control EST group (1.68) (P = 0.001).

Litter Characteristics

In the study, litter characteristics were found to be similar throughout the experiment groups, as expected. Litter pH was found as 7.96, 7.68, and 7.81 in low, control, and high EST groups, respectively (P = 0.665), whereas litter moisture was 28.3%, 28.1%, and 27.9%, respectively (P = 0.979).

Incidence and Severity of FPD and HD

The effects of different EST treatments on FPD are shown on Table 3. In the experiment groups, the percentage of broilers with score 1 was found as 14.5%, 13.7%, and 8.3% in the low, control, and high EST groups, respectively (P < 0.01). Scores of 2, 5, and 5 were found to be similar among the treatment groups, whereas score 2 was found to be higher in the control and high EST groups (27.7% and 29.2%) compared to the low EST group (16.9%; P < 0.01). In the study,

			FPD score (%)		
Item	Score 1	Score 2	Score 3	Score 4	Score 5
EST groups					
Low	14.5^{a}	23.4	16.9^{b}	41.8	3.3
Control	13.7^{a}	17.2	27.7^{a}	37.5	4.0
High	$8.3^{ m b}$	18.6	$29.2^{\rm a}$	40.4	3.7
SEM	1.63	2.77	2.63	2.78	0.60
Sex					
Female	12.8	19.9	24.3	39.9	3.6
Male	11.5	19.5	24.9	39.8	3.7
SEM	1.33	2.26	2.15	2.27	0.50
Source of variation			<i>P</i> -value		
EST	0.005	0.102	0.001	0.317	0.443
Sex	0.355	0.878	0.812	0.966	0.905
$EST \times Sex$	0.226	0.978	0.904	0.246	0.486

Table 3. Incidence and severity of FPD at 42 d of age of male and female broilers from different EST groups.

^{a,b}means in a coloumn without a common superscript are significantly different (P < 0.05). n: 6 replicate pens/treatment group (40 broilers/pen).

Table 4. Incidence and severity of HD at 42 d of age of male and female broilers from different EST groups.

			HD score (%)		Score 5
Item	Score 1	Score 2	Score 3	Score 4	
EST groups					
Low	21.1^{a}	56.2^{a}	16.2^{a}	$5.4^{\rm c}$	1.1^{b}
Control	6.9^{b}	25.3^{b}	27.9^{b}	36.2^{b}	3.7^{a}
High	$0^{\rm c}$	15.3^{c}	37.5°	43.4^{a}	3.8^{a}
SEM	2.02	2.38	2.23	1.71	0.80
Sex					
Female	9.2	32.1	27.7	28.9	3.2
Male	9.5	32.4	26.7	27.8	2.5
SEM	1.65	1.94	1.82	1.39	0.65
Source of variation			<i>P</i> -value		
EST	0.000	0.000	0.001	0.000	0.008
Sex	0.872	0.869	0.612	0.447	0.261
$EST \times Sex$	0.988	0.978	0.098	0.478	0.886

^{a-c}means in a coloumn without a common superscript are significantly different (P < 0.05). n: 6 replicate pens/treatment group (40 broilers/pen).

neither eggshell temperatures nor broiler sex affected the incidence or severity of FPD.

Incidence and severity of HD for the experiment groups are shown on Table 4. Eggshell temperature significantly affected the HD, whereas broiler sex had no impact. All of the broilers in the high EST group had dermatitis on the hock, while percentages of 21.1% and 6.9% of broilers had a score of 1 for HD in the low and control EST groups, respectively (P < 0.01). Scores of 2 and 3 for HD were found to be highest, with values for the broilers from the low EST group (56.2% vs. 16.2%, respectively) than control and high EST groups (P < 0.01). Significant differences were observed for score 4 and 5 of HD in EST groups (P < 0.01). A score of 4 was found to be highest in broilers from high EST groups (43.4%), and a score of 5 was higher in broilers from the control and high EST groups (3.7% and 3.8%).

Feathering Status

Feathering status of broilers from EST groups are shown on Table 5. Significant differences were observed for EST groups, but not any significant differences for broiler sex or for EST groups × broiler sex interactions were found in the study. All of the broilers in the low, control, and high EST groups were found to have a mean score 1.2, 1.3, and 1.3 for feathering status of the breast, respectively (P > 0.05), but this differences was not significant. On the other hand, feathering status of the wing, neck, back, and vent differed among the treatment groups (P < 0.05). Broilers in the low and control EST groups had a lower score for the wing (2.7 and 2.5) compared to the high EST group (2.1), whereas broilers in the low EST group had the highest score for neck, back, and vent with values of 2.6, 2.3, and 1.7, respectively. Mean feathering score was found to be highest with a value of 2.0 for the low EST group (P < 0.01).

Gait Score

The effects of EST treatment and broiler sex on gait score are shown on Table 6. Score 0 was observed in low, control, and high EST groups with values of 19.1%, 12.9%, and 5.6%, respectively (P < 0.01), whereas broiler sex did not affect the percentage of score 0. It

Table 5. Feathering status at 42 d of age of male and female broilers from different EST groups.

Item	Feathering status						
	Breast	Wing	Neck	Back	Vent	Mean	
EST groups							
Low	1.2	2.7^{a}	2.6^{a}	2.3^{a}	1.7^{a}	2.0^{a}	
Control	1.3	2.5^{a}	$2.4^{\mathrm{a,b}}$	$2.1^{\mathrm{a,b}}$	1.3^{b}	1.9^{b}	
High	1.3	2.1^{b}	2.2^{b}	1.8^{b}	$1.4^{\mathrm{a,b}}$	$1.7^{\rm c}$	
SEM	0.19	0.11	0.13	0.14	0.13	0.06	
Sex							
Female	1.2	2.5	2.4	2.1	1.4	1.9	
Male	1.3	2.4	2.4	2.1	1.5	1.9	
SEM	0.15	0.09	0.10	0.12	0.10	0.05	
Source of variat	tion			P-value			
EST	0.883	0.000	0.010	0.010	0.034	0.00	
Sex	0.485	0.236	0.537	1.000	0.227	0.862	
$EST \times Sex$	0.427	0.885	0.712	0.494	0.947	0.687	

^{a-c}means in a coloumn without a common superscript are significantly different (P < 0.05). n: 6 replicate pens/treatment group (40 broilers/pen).

Table 6. Percentage of gait score at 42 d of age of male and female broilers from different EST groups.

			Gait score (%)		
Item	Score 0	Score 1	Score 2	Score 3	Score 4
EST					
Low	19.1^{a}	$33.4^{\rm a}$	25.3^{b}	22.1^{c}	$0.0^{ m c}$
Control	12.9^{b}	24.1^{b}	30.3^{a}	28.6^{b}	4.1^{b}
High	5.6°	30.3^{a}	19.2°	32.3 ^a	$12.6^{\rm a}$
SEM	1.15	1.52	1.24	1.21	0.54
Sex					
Female	12.9	28.3	23.1^{b}	27.5	4.7^{b}
Male	12.2	30.2	26.8^{a}	27.9	6.4^{a}
SEM	0.94	1.24	1.01	0.99	0.44
Source of variation			<i>P</i> -value		
EST	0.000	0.000	0.000	0.000	0.000
Sex	0.476	0.149	0.004	0.671	0.002
$EST \times Sex$	0.053	0.401	0.000	0.011	0.001

 $^{\rm a-c}{\rm means}$ in a coloumn without a common superscript are significantly different (P < 0.05).

For evaluaiton of walking ability, each broiler was individually monitored for walking ability.

n: 6 replicate pens/treatment group (40 broilers/pen).

was found that a higher value of score 1 was found in broilers from the low and high EST groups (33.4% vs. 30.3%; P < 0.01). Significant interactions between EST treatment and broiler sex were observed with scores 2, 3, and 4 in the study (Figure 1A, B and C). Score 2 was found to be higher male broilers from the low EST group, male broilers from the control EST and female broilers from the control EST (P < 0.01). Also, score 3 was the highest in female broilers from the high EST group (P < 0.05), while score 4 was the highest in male broilers from high EST group (P < 0.01).

DISCUSSION

In the present study, changes in the EST clearly affected the broiler performance, as recently reported by Lourens et al., 2005, 2007; Meijerhof, 2009; Ipek et al., 2014. Hulet et al. (2007) demonstrated that different eggshell temperatures affected significantly the feed conversion ratio of broilers of 44 days of age. They found feed conversion with values of 1.91, 1.86 and 1.87 at 37.5° C, 38.6° C, and 39.7° C, respectively. Similarly,

in our study a higher FCR was observed in the low and high EST groups, which means that EST fluctuations affect also feed efficiency of broilers.

FPD and HD are painful for broilers and negatively affect walking ability, performance, and welfare conditions. In many countries, mainly Europe and the United States, FPD is used as a criteria for evaluation of welfare as it causes pain and discomfort (Algers and Berg, 2004; Berg, 2004; Haslam et al., 2006, 2007; Bilgili et al., 2009). FPD and HD can develop with discolouration of the skin and then ulceration, scrabs, and hyperkeratosis of the region (Meluzzi et al., 2008; Shepherd and Fairchild, 2010). As it appears, development of these lesions is related to skin strength which is associated with the dermis and epidermis (Kafri et al., 1986). Some known factors affecting the FPD are genotype, sex, live weight, stocking density, litter quality, season, climatic conditions, diet content and quality, type of drinker, and types of feeder (Haslam et al., 2007; Shepherd and Fairchild, 2010). Recently however it has been reported that one of the predisposing factors for development dermatitis could be the incubation conditions,



Figure 1. (A) Percentage of score 2 at 42 d of age of male and female broilers from different EST groups (n: 6 replicate pens/treatment group (40 broilers/pen)). (B) Percentage of score 3 at 42 d of age of male and female broilers from different EST groups (n: 6 replicate pens/treatment group (40 broilers/pen)). (C) Percentage of score 4 at 42 d of age of male and female broilers from different EST groups (n: 6 replicate pens/treatment group (40 broilers/pen)).

especially temperature. Da Costa et al. (2014) reported that a low incubation temprature (36.9°C) during the first days of incubation negatively affected development of the dermis and could cause a reduction in collagen content of the foot pad skin. In the study, it was found that the incidence and severity of FPD and HD were higher in the low and high EST treatments compared to the control EST group. Variation in the severity of FPD and HD in the low and high EST groups may be affected by the exposure time temperature treatment.

It has also been reported that male broilers show a higher susceptibility to lesions than females because of their higher live weight gain so that the incidence of lesions in males is higher than females (Bilgili et al., 2006). Contrarily, Harms et al. (1977) and Kjaer et al. (2006) concluded that lesions are seen to have a higher incidence in females compared to males, because female skin is thinner and contains less protein and collagen than males, so that females will have a higher susceptibility to lesions (Harms et al., 1977).

Feathers are of vital importance for poultry due to their role in temperature regulation and protection of the skin (Pilecco et al., 2011). As previously mentioned, it is reported that a deficiency in feather development precipitates some skin problems including breast blisters, skin scratches on various part of body, or dermatitis on the breast (Elfadil et al., 1996). These problems could cause microbial inflammation on the skin as well as pain, which consequently results in worse broiler performance, welfare status, and carcass quality (Pilecco et al., 2011). It is known that the formation of feather begins during the embryo development stage, so all of the factors affecting embryo development have the potential to impact feather development, especially the incubation temperature (Lourens et al., 2005; Ipek et al., 2014). Recently, it was reported that fluctuations in incubation temperature affected feathering according to the body region of the broiler (Dahlke et al., 2008). In a study performed by Scott et al. (2015), it was found that a low incubation temperature increased the number of feather follicles in the thigh, whereas a standard incubation temperature increased the number of follicles in the breast. The dermis and epidermis relationship during incubation could affect the feathering status during the growing period, especially under the same management conditions. In our study, the results showed that the feathering status differed according to body part including the wing, neck, back, and vent. Broilers from the low EST treatment group had the highest score for feathering. Correspondingly, the general mean score for feathering was found to be the highest for broilers from the low EST treatment group.

During the incubation period, variations in incubation temperature affect bone development, including the growth of long bones (Brookes and May, 1972), so that the incidence and severity of leg disorders, walking ability, and locomotion issues may also be affect by such variations. During the growing period, leg disorders and defects in walking ability result in a reduction of broiler performance including live weight, weight gain, and feed consumption and such defects also increase pain and discomfort and subsequently represent a deterioration of welfare status. In a study performed by Hammond et al. (2007), it is reported that high incubation temperature $(38.5^{\circ}C)$ resulted in an increase of the tibia and tarsus length. Oviedo-Rondon et al. (2009b) found that broilers obtained from a standard temperature profile and controlled conditions in a single stage incubator had better gait scores than broilers exposed to uncontrolled incubation temperature. In this study, unexpectedly it was found that a higher of gait score was observed in broilers from the control EST treatment group compared to the low and high EST groups. This can be explained by the fact that the live weight of broilers from the control EST group was higher. On the other hand, the incidence of a gait score of 3 and 4 were found to be closer for broilers from the control and high EST treatment groups. Male and female broilers from the high EST group had a higher gait score, which means that the high temperature during the incubation period affected the walking ability due to a variation in bone development.

In conclusion, small changes in the EST during incubation could occur in practical conditions due to large capacity incubators and the high growing rate broiler embryos. It is known that suboptimal incubation conditions, especially incubation temperature, have the potential to affect embryo development, and subsequently broiler growth performance, health, and welfare status. Thus determination of embryo physiologic requirements is needed and has vital importance to obtain maximum performance and profitability. Therefore, optimum conditions should be provided during both the incubation period and the post-hatch growing period and careful attention must be paind to critical issues such as incubation conditions, managemet practices, and feeding techniques, all of which could be manipulated to meet the demands of broilers. Detailed studies must be performed using different variables under practical conditions.

ACKNOWLEDGEMENTS

This study was financially supported by the Scientific Research Project Council Of Uludag University (Project Number KUAP(Z)-2012/14).

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