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The effects of Environmental Enrichment on Some Physiological and Behavioral Parameters of Broiler Chicks

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ABSTRACT

This study aims to examine the effects of environmental enrichment (EE) practices on some physiological and behavioral parameters of broiler chicks. A total of 280 one-day old Ross 308 mixed-sex broiler chicks with an average initial body weight of 44.5±0.37 g were used in a 42-d trial. Broiler chicks were randomly allocated to 20 pens composed of 14 birds in 1x1.4m sized floor area. Each of the EE treatment consisted of 5 replicate pens (70 chickens per treatment). The treatments were 1) control; 2) perch for EE; 3) ball for EE; 4) mirror for EE; and 5) dust for EE. Final body weights, tonic immobility test results, and rectal temperatures were recorded. Spleen, liver, and bursa fabricius weights were measured. The blood biochemistry of birds was analyzed at the end of the experiment. Besides, gait score test was applied on the 42nd day of experiment. Body weight gain, lymphoid organ weights and rectal temperature values of broilers were not affected by EE treatments on the 21st and 42nd days of the study. At the end of the study, WBC values, tonic immobility values and gait scores of the control groups were found higher than those of the EE groups. HCT and PLT values of the ball groups were found lower than the other groups, concluding that EE improved the welfare of birds.

INTRODUCTION

Environmental enrichment (EE) is defined as using various objects to improve life quality and normal behavior expression of animals which are kept in cages or in a limited space (Belz *et al.*, 2003). EE is the addition of biologically relevant stimuli to animals' environment to encourage natural behaviors (Leone & Estevez, 2008). The visuals, audio sounds, structures, plastic materials, and smells are some basic enrichment strategies in poultry housing systems (Adeniji, 2012). EE programs are considered to have an important potential in terms of both farm management and animals' health, productivity, and welfare (Estevez, 2009). Besides, it was reported that these applications decreased fear sensitivity without having any negative effect on birds' growth performance and stress (Altan *et al.*, 2013). However, it should be considered that mirror application can address potential differences in reactive and proactive aggression, although caution should be taken when using the application as some birds are able to self-recognize (Branch *et al.,* 2015). On the other hand, dust bathing is important behavior for chickens that are willing to work for access to litter for both foraging (Widowski & Duncan, 2000). Fernandes *et al*. (2015) stated that birds enjoyed red feed particles in pecking behavior. Perches offer birds the possibility to use the third spatial dimension, and can increase movement and exercise while jumping on and off of them as birds move around (Bizeray *et al.,* 2002).

Fear is a significant indicator of stress for poultry, and Tonic Immobility (TI) is used to determine fear situation for poultry as a reliable criterion (Jones, 1986). When bird is stimulated for TI, more coward ones stay motionless longer time compared to those which fear less sensitively (Taskin, 2009). Besides, fear negatively affects the welfare level of broilers and accepted as physiological and behavioral reaction when animals perceive any signals such as sudden noise as a danger (Cockrem, 2007).

Environmental enrichments improves immunity in stressed animals (Benaroya-Milshtein *et al*., 2004; Meijer *et al*., 2007; Arranz *et al.,* 2010). It is well known that stress factors affect poultry production. When poultry are exposed to stress, the consumption of glucose raises substantially, and liver gives glycogen to blood in order to balance the glucose level in blood (Garriga*et al.,* 2006). It was determined that cold stress raised glucose level in blood to 205 mg/dl from 189.8 mg/dl, raised total protein, cholesterol, triglyceride levels to 3.3 g/dl from 3.5, and went down to 110.8 mg/dl from 114.8, to 68.2 mg/dl from 72.0 in a study conducted about 42-old-day chickens (Daneshyar*et al.,* 2009). Selective breeding in broilers resulted in an increase in performance for many years affecting the welfare of broilers negatively. Fast growing broilers kept under continuous light lose locomotor activity with age due to the significant decrease in time used for this activity. Lower locomotor activity and the increase of growth rate significantly cause gait anomalies and leg problems (Seremet, 2007). It should be taken into consideration that new wood shavings lessen locomotion problems (Almeida Paz *et al*., 2010). Poor leg health may cause a decline in locomotor activity as well. Perch usage enhances the welfare of laying hens; on the other hand, the effect of perch accessibility on broiler rearing systems remains ambiguous because there is not sufficient research on this subject (Jiao *et al*., 2014). Therefore, the aim of the current study is to investigate the effects of environmental enrichment tools (perch, ball, mirror, dust) on some physiological (growth, lymphoid organ weights, rectal temperature and blood biochemistry) and behavioral (tonic immobility and gait score) parameters of broiler chicks.

MATERIAL AND METHODS

This study was conducted with the permission (11.07.2013/1/4) of Ahi Evran University Animal Experiments Local Ethics Committee. In this study, 280 one-day-old Ross®308broiler chicks both male and female in mixture were used. After all chicks were weighed, they were reared in cages to 4 days of age in a climate-controlled room, and taken to pens in a conventional poultry house with yellow curtains on the 5th day. The broiler chicks were randomly distributed in 20 pens with 1.4 m² of floor area, at a population density of 10 birds/m^2, and each treatment consisted of 5 pens (70 birds per treatment).

The pens used during experiment were made from wooden material, had a door at the front, and metal wire at all four sides. A 10-cm high wood shaving litter material was bedded on the pen floor. In the first week, the chicks were provided with a temperature of 33^{o}C, the temperature was then reduced by 3^{o}C every week and a temperature of 18^{o}C was attained at the end of the sixth week. The experiment was carried out in April and May, in 2015. Traditional fluorescent light (23:1) was applied in the cages used in the experiment. Feed and water were provided ad libitum. The chicks were fed with same commercial broiler starter (230 g CP and 3.100 kcal of ME per kg) from 1-d until 21-d and grower (200 g CP and 3.100 kcal of ME per kg) from 22-d until the end of the experimental period. Nutrients and energy concentrations of experimental diets met or exceeded the minimum requirements of NRC (1994). Strict sanitation practices were applied throughout the experiment. No bird was sacrificed during the study.

Environmental enrichment consisted of objects designed to encourage pecking and locomotor activity in the pen. The design of the objects was modified based on the results of the previous studies on broiler chickens. The treatments included control, perch for EE, ball for EE; mirror for EE; and dust bath for EE (Fig. 1).

Enrichments objects (mirrors and balls) were suspended from overhead wires in such a way that they dangled from the pen ceiling (Jones *et al*., 2000). Objects at 30 cm from the ground were suspended by 5 cm upgrade in each week at the end of the 42th day. The properties of objects were as follows:

A double-faced mirror (20 x 10 cm). The ball was a red one, made of plastic with a diameter of 10 cm. The perch was 120 cm long with a wooden structure which included a horizontal and a sloped (13°) section. The dust bathing pool was 10 cm deep, 40-cm diameter plastic black container. There was a small grain of sand in it.

Chicks were weighed weekly and their body weights were taken. The increase of weekly body weight was calculated as subtracting weighing week before the weighing applied. Rectal temperatures

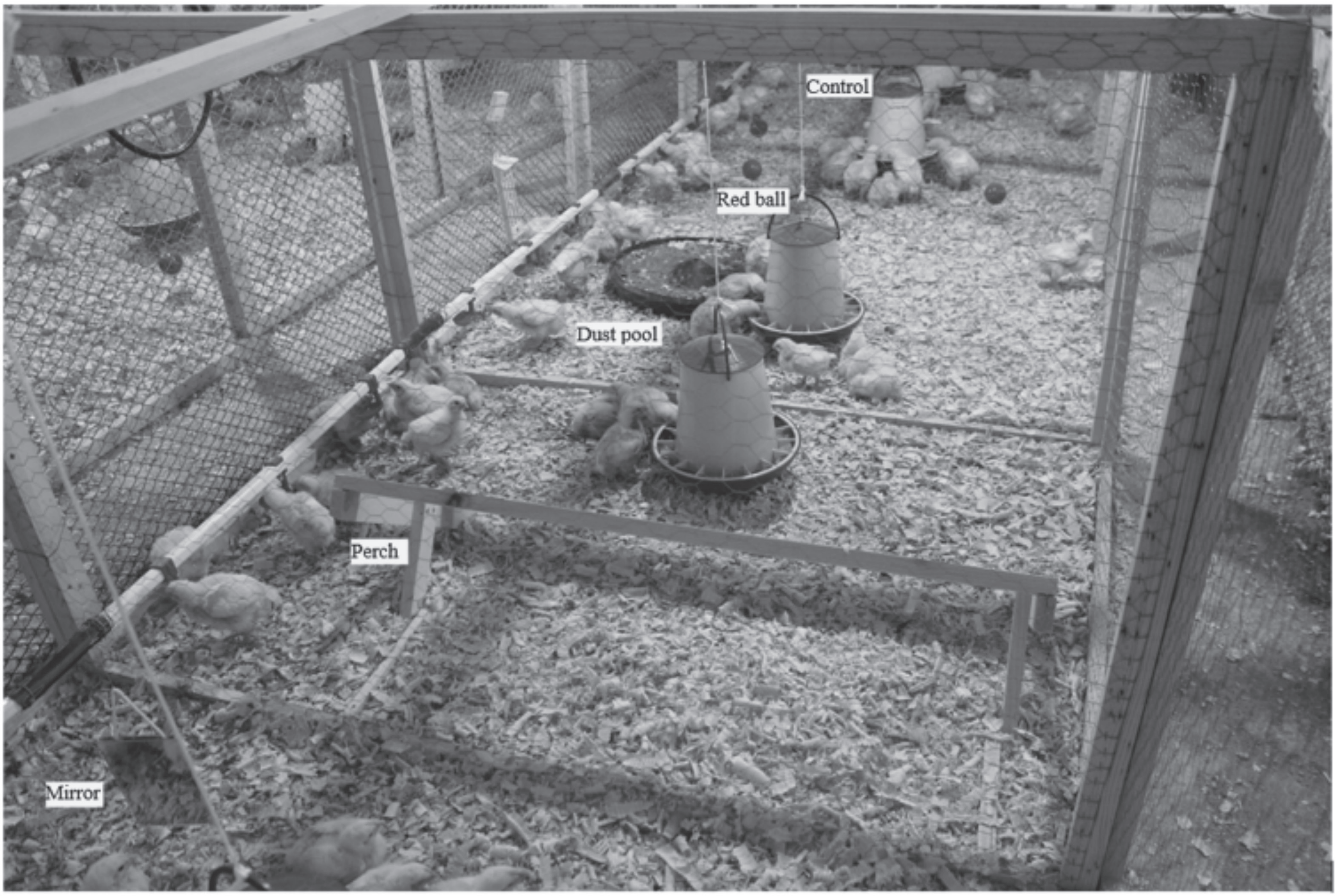

Figure 1 – Enriched pens with perches, dust pools, mirrors and red balls

of the fifteen birds from each group were measured at 06:00 h, 14:00, and 18:00 h of the 21st and the 42nd day by one observer because these hours refer to daily climate changes during the day (Sinkalu *et al*., 2015). For this purpose, a digital clinical thermometer (Hartmann, Paul Hartman AG, Heidenheim, Germany), was inserted approximately 2 cm for the 21st day and 5 cm for the 42nd day into the cloaca and in direct contact with the mucosal wall. The value was recorded after the thermometer gave an alarm sound, indicating that the reading had been stabilized (Sinkalu *et al.,* 2015). At the end of the experiment, 15 birds in each group were slaughtered humanly to get lymphoid organ (spleen, liver and bursa fabricius) weights

The tonic immobility (TI) test was applied as Jones & Faure (1980) reported. Animals whose TI time was longer were evaluated as more passive, referring to fear while those with shorter TI period were evaluated as being more active and fearless. The TI test of birds was conducted in a separate room within the same building on the 21st and 42nd days. TI induced as soon as the chickens were carried to the test room by restraining them on their right side and wings for 15 seconds (s). The observer was 1m away from the test table and waited without noise. A chronometer was used to record latencies until the chicken stood up itself (Taskin, 2009). If the chicken righted itself in < 10 s, it was recaptured and the procedure was repeated. If TI was not induced after 3 trials, the TI duration was recorded as zero. The maximum duration of TI allowed was 600 s (Benoff & Siegel, 1976). 25 chicks were used for each treatment on the 42nd day (06:00 h, 14:00, and 18:00 h) in our experiment.

The evaluation was based on a gait scoring system developed by Kestin *et al.* (1992). The conducted test is as follows: The chickens were induced to walk for a distance of 1 m on a flat surface containing wood shavings, simulating the broiler litter conditions found in the broiler house. The methodology consisted of subjective observations that allowed attributing a score on how the bird walked (Alves *et al.,* 2016b). According to this scoring, gait characteristics were regulated as 0 was normal, and as 5 was the worst.

Blood samples (3 ml per bird) were taken from the wing venations of 15 chicks having equal body weight from each group on the 42nd day of the experiment and centrifuged for 6 minutes at 3000 speed, and separated into serums (Arctander, 1988). Separated samples were kept at -80°C for glucose, triglyceride, cholesterol, and total protein analyses. After that these analyses were applied with clinical chemistry analyzer (BS-400 Mindray, China). Besides, cell numbers and ratios in blood were determined as analyzing hematological analysis from obtained samples at auto hematology analyzer (BC-5380 Mindray, China). However, stress hormones such as corticosterone and cortisol were not analyzed.

This study was planned on a randomized design. The statistical analyses were performed using the SPSS 16.0 (SPSS Inc., Chicago, IL, USA) software package (2007). The results were expressed as means ± S.E.M. Significant differences were determined using protected Tukey test ($P \leq 0.05$) after ANOVA.

RESULTS AND DISCUSSION

Body weight values were found as 716.10 g, and 2,573.12 g on the 21st, and 42nd day in control groups, respectively (Fig. 2). Final body weight of control groups was higher compared to other groups without statistical significance. Spleen, liver, and bursa fabricius weights of the control groups were found as 0.67 g, 21.26 g and 1.53 g with 2.87 g, 59.13 g and 3.87 g respectively, on the 21st and 42nd days without statistical significance compared to the EE treatments (Table 1). Rectal temperature values of the control groups were found as 40.95°C and 40.75°C respectively, in the third and sixth weeks without statistical significance compared to the EE treatments (Fig. 3).

According to TI responses for the 21st day of trial, TI induction trial attempts number of the perch groups (2.12) was found higher than that of the other groups (Fig. 4). While ball groups' TI duration (Fig. 5) was found as 165.62 s which was higher than control groups, dust groups' duration was found lower (100.50s) than that of the control groups ($p<0.05$). According to TI responses on the 42nd day, induction trial attempts for TI of the control groups (2) was found higher than the other groups. Duration of the dust groups was found lower than the other groups for the 21st and 42nd days. Duration of the mirror groups was higher than the other groups for the 42nd day ($p<0.05$).

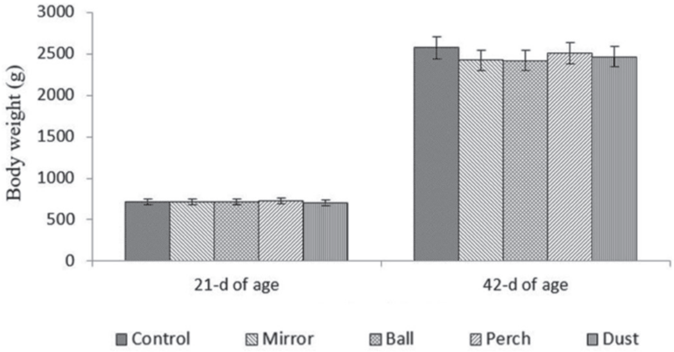

Figure 2 – Body weight (g) in 21-d and 42-d of age.

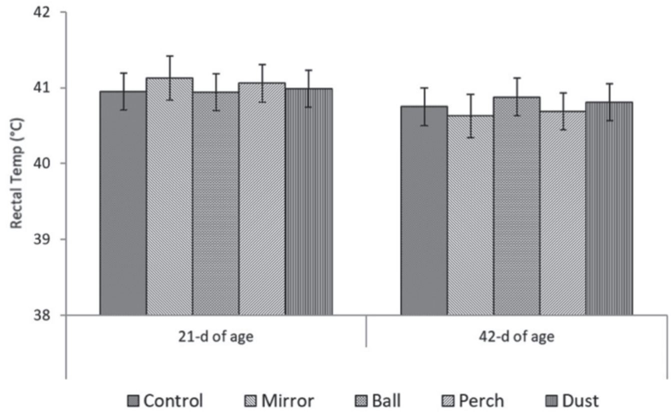

Figure 3 – Rectal temperature (°C) in 21-d and 42-d of age.

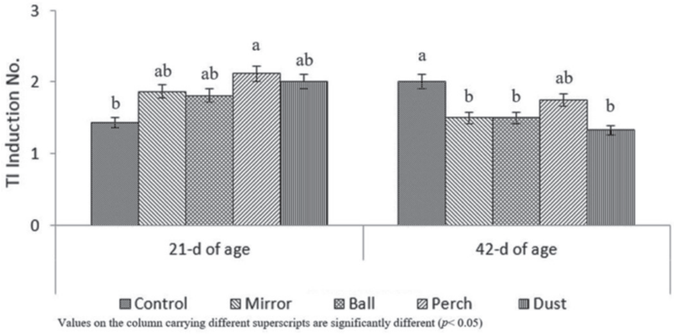

Figure 4 – TI induction number in 21-d and 42-d of age.

Table 1 – Weight of spleen, liver and bursa fabricus (g) in 21-d and 42-d of age.

Treatments	Spleen (g)		Liver (g)		Bursa fabricus (g)	
	21-d of age	42-d of age	21-d of age	42-d of age	21-d of age	42-d of age
Control	0.67 ± 0.28	2.87 ± 0.46	21.26 ± 3.65	59.13 ± 10.49	1.53 ± 0.56	3.87 ± 1.11
Mirror	0.64 ± 0.16	2.81 ± 0.43	22.24 ± 2.76	62.42 ± 12.73	1.41 ± 0.34	3.89 ± 1.29
Ball	0.61 ± 0.22	2.98 ± 0.56	21.70 ± 2.53	59.61 ± 11.84	1.35 ± 0.60	3.90 ± 0.91
Perch	0.61 ± 0.16	2.54 ± 0.22	21.14 ± 2.02	59.75 ± 11.46	1.35 ± 0.39	4.04 ± 1.75
Dust	0.81 ± 0.32	2.88 ± 0.45	21.42 ± 2.80	64.77 ± 12.45	1.70 ± 0.57	3.78 ± 1.28

All values are means ± SEM.

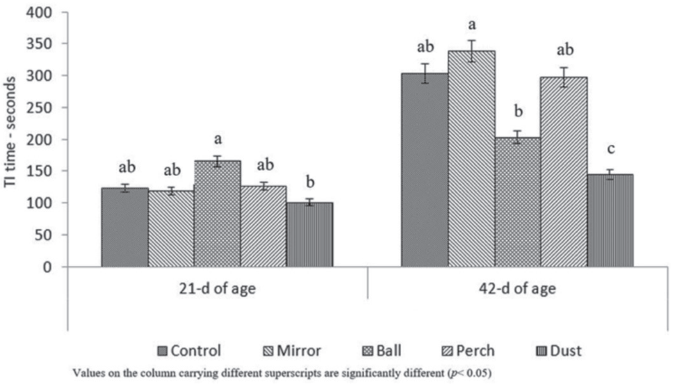

Figure 5 – TI durations (seconds) in 21-d and 42-d of age.

The perch group had a serious effect on gait score among the EE applications (Fig. 6). The lowest effect was observed in the control groups ($p<0.05$). Gait scores of the perching group were the lowest while the control group's was the highest. This statement can be explained as follows: bad walk gets high points. So, we can say that EE has improved walking ability.

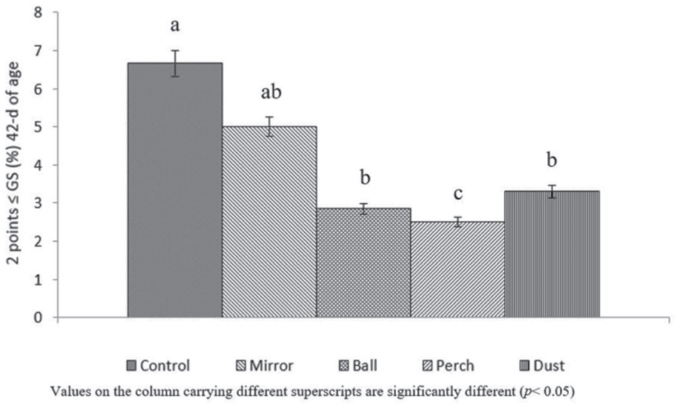

Figure 6 – Two points ≤ GS (%) in 42-d of age.

Blood biochemistry of 42-d-old experimental chicks are presented in Table 2. The highest cholesterol values were determined for mirror (2.45±0.09 mmol/l), perch (2.42±0.11 mmol/l) and ball (2.34±0.08 mmol/l) groups respectively. The highest total protein ratio was determined for the dust group (39.63±1.27 g/l) and the lowest for the perch group (34.15±1.76 g/l) ($p<0.05$). White blood cell value of the control groups (28.23±0.14 $x10^3/mm^3$) was found higher than the EE groups as a result of hemogram analysis. Ball groups' haematocrit count (%35.21±0.99) and platelet values (0.19±0.02 $x10^8/l$) were found lower than those of the other groups ($p<0.05$).

Simsek *et al.* (2009) determined body weight of the control group as 2291g in their study at the end of the 42nd day. In the same study, body weight of the EE groups was determined as 2231 g at the end of the 42nd day. Bizeray *et al*. (2002) determined that EE consisting items such as perch and barrier did not affect body weight averages of the broilers determined in the 3rd and 6th weeks in their study. Diktas *et al.* (2015) did not find statistically significant differences in terms of organ weights of the broilers which were developed slowly at different growth systems in their study. The present study results were consistent with those of the studies that have shown EE has not significantly affected body, and organ weights for broilers (Seremet, 2007; Simsek *et al*., 2009; Bizeray *et al*., 2002). In this study, it was observed that body weights of the treatment groups before slaughtering on the 42nd day were similar to those of the control groups. Being coherent with the present study, it was determined in the previous studies that body weight of broiler chicks was between 2380 g (Kaynak *et al*., 2010) and 2356 g (Seremet, 2007).

Eleroglu *et al*. (2015) determined rectal temperatures of female and male chicks measured in the sixth week were as 41.7 °C and 41.8 °C respectively in their study without showing any difference between sexes. Altan *et al.* (2000) determined rectal temperature value

Table 2 – Blood biochemistry of experimental chicks at 42-d old.

	Control	Mirror	Ball	Perch	Dust
Glucose (mmol/l)	12.56±0.25	13.33±0.28	14.40±0.35	13.20±0.32	14.54±0.21
Cholesterol (mmol/l)	1.90±0.17^c	2.45±0.09^a	2.34±0.08^b	2.42±0.11^a	1.98±0.09^c
Triglyceride (mmol/l)	1.09±0.06	1.06±0.02	1.10±0.05	0. 93±0.08	0.97±0.07
Total protein (g/l)	38.80±1.20^b	37.26±1.49^b	36.66±1.36^b	34.15±1.76^c	39.63±1.27^a
RBC ($x10^{6)}$	2.71±0.06	2.92±0.04	2.64±0.07	2.81±0.06	2.95±0.08
Hb (g/dl)	14.20±0.46	15.14±0.39	13.42±0.61	14.23±0.54	14.17±0.63
HCT (%)	37.16±1.09^a	38.24±1.43^a	35.21±0.99^b	39.27±0.86^a	38.31±0.94^a
MCV (fl)	128.27±6.17	132.64±6.35	133.74±7.28	129.29±7.36	130.36±6.49
MCHC (%)	38.17±1.83	39.27±2.30	39.43±2.62	38.55±1.96	38.24±1.43
MCH (pg)	50.10±3.21	51.00±3.24	51.40±3.09	50.20±3.41	50.30±2.74
WBC ($x10^3/mm^3$)	28.23±0.14^a	25.41±0.16^b	25.36±0.19^b	26.43±0.14^b	26.46±0.15^b
PLT ($x10^8/l$)	0.29±0.03^a	0.26±0.02^a	0.19±0.02^b	0.24±0.04^a	0.28±0.03^a

All values are means ± SEM. Values in the same row carrying different superscripts are significantly different ($p<0.05$)

in control groups in the sixth week as 40.71 °C. In another study, when the room temperature of control birds were maintained at 21 °C for 8 weeks, the similar rectal temperatures were obtained (Xie *et al*., 2015). The current findings are in agreement with the mentioned studies.

Wang *et al*. (2013) found that the broilers with a short TI time grew faster and reached higher body weights than the broilers with a long TI duration. Liver and breast weights were also affected from TI duration. However, short TI duration with a high breast weight caused lower liver weight compared to body weight (P=0.053). The present results were compatible with the study of Turkyilmaz *et al*. (2011) but were non concordant with the studies of Fidan *et al.* (2014) and Wang *et al.* (2013). This may result from the differences of treatment. Sinkalu *et al.* (2016) found that the highest numbers of induction trial attempt were 2.13 ± 0.34 obtained in 12D:12L cycle and they were recorded at 13:00 h when the broiler chickens were 8 weeks old. Their findings are in agreement with the result obtained for the perch group in this study.

Skrbic *et al*. (2009) determined the gait score value as 0.68, Ravindran *et al*. (2004) as 1.92, De Jong*et al.* (2014) as 2.10. The present gait score was higher than the findings of Skrbic *et al*. (2009) and lower than the findings of others. Alves *et al.* (2016a) determined the gait score value (2<GS) as 5.2%. The obtained EE results in the present study were lower than findings. The values of our control group were higher than in their results. This situation may have been caused by the use of rice husks as the litter material in their study. All in all, this methodology will be useful tool to assess how broilers walk (Alves *et al*., 2016b).

Fidan *et al.* (2014) determined glucose as 12.81 mmol/l, cholesterol as 3.02 mmol/l, triglyceride as 1.01 mmol/l and total protein as 31.53 g/l at the end of their study on broilers. Aslan *et al.* (2005) determined glucose as 2.07 g/L and cholesterol as 1.86 g/L in the 4th week while glucose was 2.11 g/L and cholesterol was 1.92 g/L in the 8th week. Toghyani *et al.* (2011) found total protein as 3.4 g/dl, triglyceride as 50 mg/dl, and cholesterol as 156 mg/dl. Pandurang *et al*. (2011) obtained glucose as 174.17 mg/dl and total protein values as 6.03 g/dl. Abdelrahman *et al*. (2012) determined glucose as 277.3 mg/dl, total protein as 2.50 g/dl, cholesterol as 224.7 mg/dl and triglyceride as 129.1 mg/dl. Wang *et al.* (2013) found that total protein ratio and serum cholesterol ratio were high for treatment group which had perch and sand bath. Turkyilmaz *et al.* (2011) reported that cholesterol and glucose levels, and total protein ratio of stress raised broilers decreased with the increase of stress. No significant difference was not reported between cholesterol, triglyceride, glucose levels and plasma's total protein content in other studies (Seremet, 2007; Diktas *et al*., 2015).

Blood biochemistry is affected by several factors, such as season, age, breed, nutrition and stress (Michael *et al.,* 1992; Davis *et al*., 2000). Although there are limits with reference values in blood, it has been found that the differences occurring in the blood may be caused by factors such as stress of blood collection and applied EE objects may have influenced the activity of animals. This study results were consistent with the findings of Seremet (2007) and Diktas *et al.* (2015).

Atmaca *et al*. (2012) determined the values of control group's hemogram analysis result were erythrocyte (RBC) as 2.42*10^6/mm^3, hemoglobin (Hb) as 11.24 g/dl, hematocrit (HCT), as 32.43%, Mean Corpuscular Volume (MCV), as 135,61 fL, Mean Corpuscular Hemoglobin (MCH), as 47.6 pg, Mean Corpuscular Hemoglobin Concentration (MCHC) as 35.16%, leucocyte (WBC)as 31.43*10^3/mm^3. Fidan *et al*. (2014) determined Hb as 8.26 g/dl, HCT as 23.57% at the result of control group's hemogram analysis. Aslan *et al.* (2005) found RBC, 2.33*10^{12}L, Hb, 85 g/L, WBC, 38,5*10^9/L, PLT, 0.23*10^8/L at the 4th week according to hemogram analysis. They determined values as RBC, 2.34*10^{12}L, Hb, 94 g/L, WBC, 41,7*10^9/L, PLT, 0.29*10^8/L at the 8th week of the hemogram analysis result respectively at the same study. Toghyani *et al*. (2011) determined RBC, 1.97*10^9/L, WBC, 22,8*10^3/L, Hb, 11.4 mg/ml, HCT, %25, MCV, 130 fL, MCH, 58 pg and MCHC, 44,9% in their study. Abdelrahman *et al.* (2012) determined as WBC, 24.81*10^3/L, RBC, 2.80 *10^{6}L, Hb, 11 g/L, MCV, 113.21 fL and MCH, 34.73 pg in their study on broilers. Current findings was similar to those of Atmaca *et al.* (2012), Fidan *et al.* (2014), Aslan*et al.* (2005) and Toghyani *et al*.(2011).

Chickens can behave in accordance with their basic needs in the environment that they are grown. Instead of monotone setting, poultry can be stimulated to display their basic behaviors via EE. Thus, animals' welfare level is expected to increase without causing any negative effect on their performances. Taken together, these findings provide important evidence that EE applications does a potentially positive contribution to the welfare of broiler chicks. To conclude, the environmental enrichments (ball, mirror, dust and perch) improve welfare of the birds without affecting the growth performance and lymphoid organ weights.

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