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Effect of breeder age and lighting regimen on growth performance, organ weights, villus development, and *bursa of fabricius* histological structure in broiler chickens

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ABSTRACT: This study was carried out to investigate the effect of breeder age and lighting regimen on performance, some organ weights, villus development, and *bursa of fabricius* histological structure in broiler chickens. A total of 384 one-day-old chicks were obtained from two Ross broiler breeder flocks at 32 (young;Y) and 49 (old, O) weeks of age. Chicks from each breeder age were reared under 18 h light : 6 h dark (18 L : 6 D) (control; CL) or 14 L : 4 D : 2 L : 4 D (split darkness, SD). Body weight, feed intake, feed conversion ratio, and mortality were measured weekly during the experiment. At 21 days of age, liver, heart, spleen, and *bursa of fabricius* weights were recorded, gastrointestinal tract and jejunum lengths were measured, and histomorphometry of villi and *bursa of fabricius* structure were investigated. Interaction between breeder age and lighting regimen was observed, where Y-CL chicks had the lightest body weight from 7 to 35 days ($P < 0.05$). Neither breeder age nor lighting regimen influenced feed conversion ratio. SD chicks had longer ($P < 0.05$) gastrointestinal tract and jejunum, and wider villus in comparison to CL chicks. Lower relative spleen weight was observed in CL chicks compared to SD ones ($P < 0.05$). It was concluded that split darkness lighting regimen could be used for broiler chickens from young breeders to improve live body weight without affecting feed conversion ratio.

Keywords: photoperiod; body weight; gastrointestinal tract; heart; lymphoid organs; mortality

INTRODUCTION

Lighting regimen is an important management tool in commercial broiler chickens production. Broiler chickens have been reared under 23 h light (L) : 1 h dark (D) lighting regimen to maximize growth rate, allow maximum feeding time and conscious feed consumption (Buyse et al. 1993, 1996; Lien et al. 2007). However, near-continuous lighting regimens may cause many welfare related problems (Gordon 1994; Gordon and Tucker 1997). From June 2010, new EU animal welfare regulations included stopping the use of lighting regimen with day length longer than 18 h to improve broiler welfare. Therefore, several different types of photoperiods have been tested to decrease susceptibility to metabolic diseases, skeletal disorders, and increase tibial breaking strength (Renden et al. 1996; Ingram et al. 2000; Sanotra et al. 2002; Lewis et al. 2009; Schwean-Lardner et al. 2012, 2013). Moreover, lighting regimens maintain the vitality of anatomical stress indicators such as liver (Onbasilar et al. 2007; Bayram and Ozkan 2010) and *bursa of fabricius* and spleen, which are main organs that could refer to the immunological status as well (Pope 1991; Heckert et al. 2002; Blahova et al. 2007; Ahmed and El-Ghamdi 2008).

Council Directive 2007/43/EC on the protection of chickens kept for meat production recommended at least 6 h of light in total, with minimally 4 h of uninterrupted darkness per 24 h. Schwean-Landner et al. (2009) indicated that split darkness (SD)

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periods increased growth rate when the darkness period was 9 h but not 6 h. Duve et al. (2011) reported higher weight gain and feed intake for broiler chickens reared under 8 h of split darkness compared to those reared under 8 h of continuous darkness. Higher overall feeding activity with higher crop content was also reported for broiler chickens under continuous darkness than those under split darkness.

Although effect of breeder age on broiler chickens performance is very well documented, there are some inconsistencies among studies in the literature. Heavier body weights were reported for broiler chickens from older breeders compared to those from younger ones with similar feed conversion (Ulmer-Franco et al. 2010; El Sabry et al. 2013). While, Hulet et al. (2007) found no differences in the body weight of broiler chickens from young and old breeders at 35 days of age. It can be questioned whether broiler chickens from different breeder ages give similar response to the lighting regimens because of their differences in body weight either at day 1 or slaughter age. Therefore, the present study aimed to investigate effect of split darkness interrupted with 2 h of lighting period on performance, morphological parameters of gastrointestinal tract, and weight of some organs of broiler chickens from young and old breeders.

MATERIAL AND METHODS

This study was performed according to the laws and regulations of care and use of animals in Turkey (license No. 5199).

Animal and rearing condition. The experiment was conducted using a total of 384 one-day-old broiler chickens of both sexes obtained from 2 Ross broiler breeder flocks aged 32 weeks (young, Y) and 49 weeks (old, O) of age. Balanced gender chicks (50 : 50) from each breeder age were weighed individually, wing banded, and randomly distributed into 6 replicate pens with a bird density of 16 birds/m^2, in two identical experimental rooms, in the same environmentally controlled house. The average weights were 40.05 ± 0.32 g for chicks from Y breeders and 49.61 ± 0.32 g for chicks from O breeders, and 45.85 ± 0.3 g for the chicks raised under CL regimen and 43.80 ± 0.3 g for the chicks under SD regimen.

All chicks received continuous lighting (23 h L : 1 h D) for the first 3 days of age. On days 4–35, chicks were subjected to 18 h L : 6 h D (control lighting, CL) or 14 h L : 4 h D : 2 h L : 4 h D (split dark, SD). Fluorescent lamps were used in the experiment, and average light intensity was approximately 20 lx/m^2 at chicks' level.

Brooding temperature was 32°C for the first 3 days of age, and then it was gradually declined to 24°C by 28 days of age. Thereafter, the temperature and humidity ranged between 22–24°C and 50–60%, respectively. Feed and water were provided *ad libitum* throughout the experimental period. Broiler chicks fed a commercial starter diet (235 g crude protein (CP)/kg; 12.00 MJ metabolizable energy (ME)/kg) for the first 14 days, a grower diet (228 g CP/kg; 12.40 MJ ME/kg) on days 15–28, and a finisher diet (221 g CP/kg and 12.90 MJ ME/kg) on days 29–35.

Parameters measured. Chicks were weighed individually and feed intake was recorded on pen basis at weekly intervals and feed conversion ratio was calculated. Died chicks were recorded daily on the pen basis. At day 21, five chicks were randomly chosen from each breeder age/lighting regimen, and then were sacrificed by cervical dislocation. Heart, liver, *bursa of fabricius,* and spleen were excised and weighed and relative weights were calculated. Samples obtained from jejunum, *bursa of fabricius,* and spleen were fixed in Bouin solution, stained with hematoxylin and eosin. Five randomly selected villi/chick were measured in three serial histological sections under light microscope XSZ-PW 146 (Proway Optics and Electronics, China) at magnification ×4. Measurements of villus height and width were performed using Sigma Scan Pro5 program (Version Pro 5.0, 2004), then villus surface area was calculated. Histological structure of *bursa of fabricius* and spleen was also examined under light microscope (magnification ×10 and ×4, respectively).

Statistical analysis. Data were analyzed using JMP statistical program of SAS (Statistical Analysis System, Version 5.0, 2003). The model used for performance, gastrointestinal measures and organs included breeder age, lighting regimen, and their interaction as main factors. Because chicks' body weight between lighting groups was significantly different at hatch, chick weight was included in the model as covariate to analyze body weight data. Duncan's multiple range test was used to separate the means when significant differences among treatment means were found. Total mortality data were analyzed by using Chi-square statistics.

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RESULTS AND DISCUSSION

Live performance. An interaction between breeder age and lighting regimen significantly affected body weight on days 7–35 (Table 1). On day 7, body weights of Y chicks under both lighting regimens were similar. Body weight of O chicks followed the same pattern. However, the chicks from O breeders under SD regimen (O-SD) had significantly heavier body weight compared to chicks from Y under SD regimen (Y-SD) (Figure 1). From day 14 to day 35, chicks from Y under CL regimen (Y-CL) had the lowest body weight compared to the other groups ($P < 0.05$) (Figure 1). These results referred to the different response of the chicks from Y and O breeders to lighting regimens and may show the significant effect of the SD regimen on enhancing the body weight of chicks from Y breeders.

Interaction between breeder age and lighting regimen was also significant for feed intake in the first half of growing period (days 1–21) ($P < 0.05$). The Y-CL chicks had lower feed consumption than Y-SD ones (1026 vs 1144 g under CL and SD regimens, respectively), while chicks from O consumed similar amount of feed under both lighting regimens (1258 and 1208 g under CL and SD, respectively) (data not shown in tables). This result is in line with the lowest body weight of Y-CL broiler chickens. However, neither breeder age nor lighting regimen affected feed conversion. Similarly, Dorminey (1971), Buyse et al. (1996), and Schwean-Lardner et al. (2012) found no difference in feed conversion ratio when broiler chicks were reared under different lighting regimens. Furthermore, feed intake and feed conversion were similar among groups on days 22–35, indicating that broiler chickens adapted to lighting schedule and modified their feeding behaviour, and consequently feed intake. Mortality was similar between breeder age and lighting regimen groups ($\chi^2 = 0.052$, $P = 0.819$ and $\chi^2 = 3.04$, $P = 0.081$, respectively).

Gastrointestinal tract and villus measurements. The current investigation indicated that breeder age did not affect gastrointestinal tract measurements (Table 2). This result is in agreement with Applegate et al. (1999), who found that breeder age did not affect duodenum or jejunum/ileum weight or length and villus height in turkey poults at day 7 of age. However, present results of villus measurements were not in line with those of Mahmoud and Edens (2012) who reported that the variation in the morphometric measurements of chicks during the early days post hatch which was associated with breeder age.

However, lighting regimen had a significant effect on the gastrointestinal tract measurements, except villus area (Table 2). Split dark chicks had longer ($P < 0.05$) gastrointestinal tract and jejunum length, as well as wider villus in comparison to CL

Table 1. Effect of breeder age and lighting regimen on body weight, feed intake, and feed conversion ratio (FRC) of broiler chickens

	Body weight (g)					Feed intake		FCR	
Day(s)	7	14	21	28	35	1–21	22–35	1–21	22–35
Breeder age									
Young	178	466[b]	948[b]	1606[b]	2325[b]	1085[b]	2156[b]	1.23	1.58
Old	174	488[a]	1012[a]	1705[a]	2501[a]	1236[a]	2458[a]	1.23	1.63
SEM	2.3	6.4	13.4	19.4	33.1	19.2	65.9	0.02	0.05
Lighting regimen									
Control	174	460[b]	960[b]	1650	2413	1142	2240	1.23	1.53
Split dark	178	495[a]	1001[a]	1662	2413	1179	2374	1.23	1.68
SEM	1.9	5.5	10.5	15.5	26.5	18.8	66.4	0.02	0.06
ANOVA									
Breeder age (BA)	0.315	0.041	0.002	0.002	0.001	< 0.001	0.006	0.893	0.524
Lighting regimen (L)	0.125	< 0.001	0.007	0.613	0.986	0.220	0.174	0.983	0.055
BA × L	0.003	0.001	< 0.001	< 0.001	< 0.001	0.0142	0.237	0.458	0.642
Chick weight	< 0.001	< 0.001	< 0.001	< 0.001	0.014	–	–	–	–

[a,b]means with different superscripts, within a trait and variable, differ significantly ($P < 0.05$)

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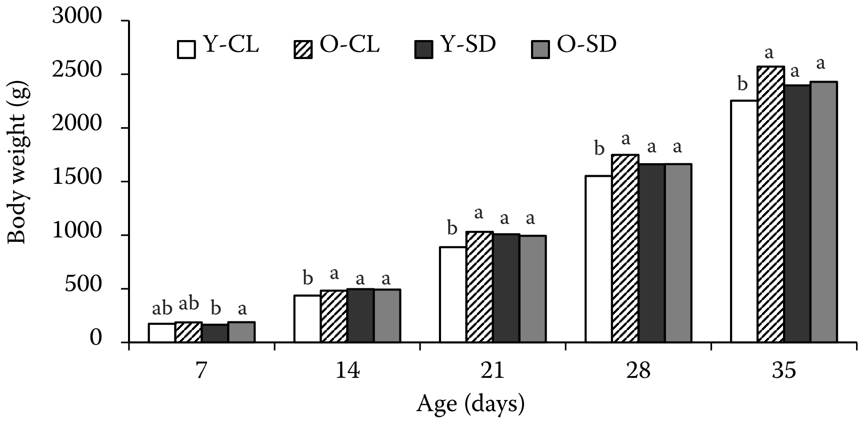

Figure 1. Effect of interaction between breeder age and lighting regimen on body weight of broiler chickens on days 1–35 of age

Y-CL = chicks from young breeders, control lighting; O-CL = chicks from old breeders, control lighting; Y-SD = chicks from young breeders, split dark lighting; O-SD = chicks from old breeders, split dark lighting

[a,b]means with different superscripts significantly differ ($P < 0.05$)

chicks. Conversely, chicks of CL group had higher villus compared to those of SD group ($P < 0.05$). Jamroz (2005) stated that longer small intestine is important for nutrient digestion and utilization especially of short chain amino acids. This may explain heavier body weight of SD chicks even though CL chicks received extra 2 h light per day for feed consumption.

Organ weights and histological structure. Results of the present study showed that breeder age had no effect on organ weights measured, which is in agreement with Maiorka et al. (2004) (Table 3). Previous studies showed an increase in liver weight (Malheiros et al. 2003; Lin et al. 2006) and decrease in spleen, *bursa of fabricius,* and heart weights under stressful conditions (Puvadolpirod and Thaxton 2000; Lin et al. 2006). There was no lighting regimen effect on relative weights of heart, liver, and *bursa of fabricius*.

Zikic et al. (2010) stated that negative histological changes in *bursa of fabricius* structure such as the follicles atrophy, increase of connective tissue, and appearance of cysts in epithelium were observed due to sound stress or progresses in broiler age. In the present study, *bursa of fabricius* histological sections showed an increase in the connective tissue in both Y-CL and O-SD broiler chickens (Figure 2). These results were consistent with finding of Abbas et al. (2008) who reported that intermittent lighting regimen activated both peripheral T and B lymphocytes proliferation and increased antibody production compared to the continuous lighting regimen. It could be suggested that lighting regimen may affect immune response

Table 2. Effect of breeder age and lighting regimen on morphological characteristics of the gastrointestinal tract (GI) and villus parameters of broiler chickens at day 21

	GI tract length (cm)	Jejunum length (cm)	Villus height (mm)	Villus width (mm)	Villus area (mm^2)
Breeder age					
Young	127	51	1.25	0.0791	0.099
Old	132	55	1.24	0.0846	0.104
SEM	4	2	0.015	0.002	0.003
Lighting regimen					
Control	121^b	50^b	1.33^a	0.076^b	0.102
Split	138^a	56^a	1.16^b	0.088^a	0.103
SEM	3.6	1.8	0.015	0.002	0.002
ANOVA (*P*-values)					
Breeder age (BA)	0.432	0.105	0.541	0.068	0.175
Lighting regimen (L)	0.009	0.026	< 0.001	< 0.001	0.871
BA × L	0.235	0.134	0.993	0.601	0.893

[a,b]means with different superscripts, within a trait and variable, differ significantly ($P < 0.05$)

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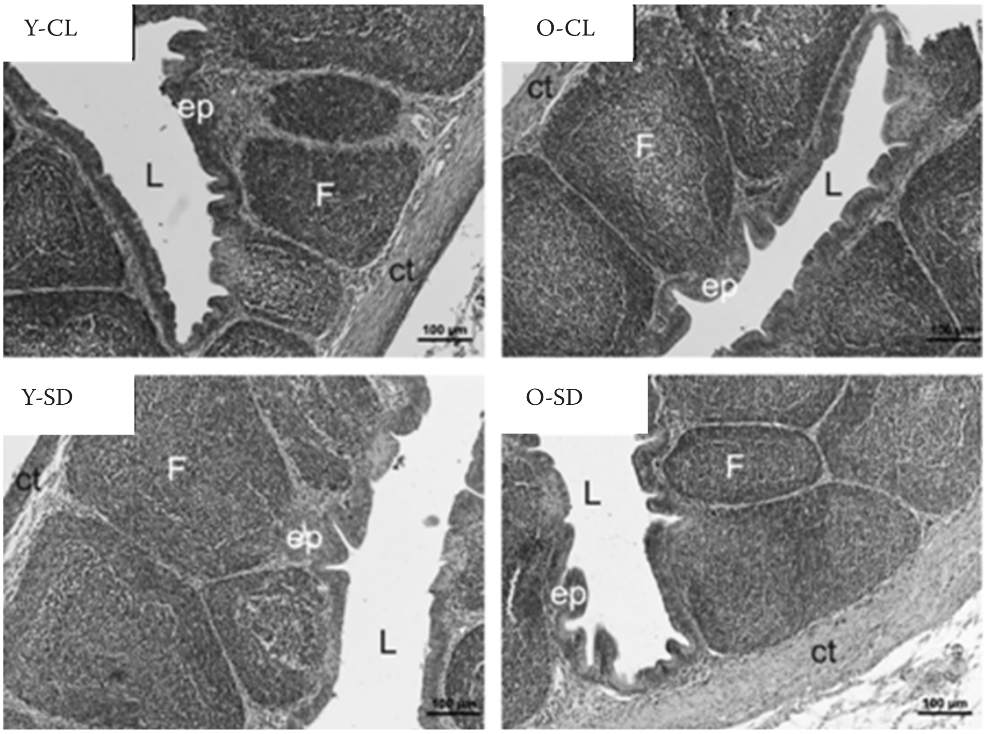

Figure 2. Histological section of *bursa of fabricius* of broiler chickens on day 21 of age

Ct = cortex, F = follicle, ep = epithelium tissue, L = lumen
Y-CL = chicks from young breeders, control lighting; O-CL = chicks from old breeders, control lighting; Y-SD = chicks from young breeders, split dark lighting; O-SD = chicks from old breeders, split dark lighting

of broiler chickens and this response may interact with breeder age.

Spleen is the most important lymphoid organ and combines the innate and adaptive immune response in a uniquely organized way. Pardue et al. (1985) and Awadalla (1998) reported lighter spleen weight after chick's exposure to environmental stressors. The results of the present study showed that spleen of CL chicks was significantly heavier than that of SD chicks. Spleen comprises

Table 3. Effect of breeder age and lighting regimen on relative weight of heart, liver, bursa, and spleen of broiler chickens at day 21

	Heart (%)	Liver (%)	Bursa of fabricius (%)	Spleen (%)
Breeder age				
Young	0.730	2.793	0.264	0.092
Old	0.705	2.730	0.257	0.095
SEM	0.022	0.08	0.018	0.009
Lighting regimen				
Control	0.730	2.656	0.249	0.105[a]
Split	0.705	2.866	0.272	0.082[b]
SEM	0.022	0.090	0.018	0.006
ANOVA (*P*-values)				
Breeder age (BA)	0.456	0.612	0.765	0.809
Lighting regimen (L)	0.437	0.098	0.371	0.015
BA × L	0.592	0.254	0.273	0.926

[a,b]means with different superscripts, within a trait and variable, differ significantly ($P < 0.05$)

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two principal compartments: red pulp, which filters the blood of foreign material and damages old erythrocytes, and white pulp, which initiates immune reactions to blood-borne antigens (Mebius and Kraal 2005). In rodents, Hernandez et al. (2013) found that red pulp expanded at the expense of white pulp due to strained stress exposure, while the histological structure of spleen of the groups in this study did not show a clear difference. Duve et al. (2011) stated that to split dark period into two periods per 4 h may not confer considerable welfare benefits to the young chicks and may affect the quality of rest, which could be related to the interruption in the melatonin rhythm (Yamada et al. 1988).

CONCLUSION

The results of the present study showed that lighting regimen could affect the response of broiler chicks due to breeder age. Even though, lighting regimen used in this experiment had no effect on live performance of broiler chicks from older breeders, SD could be used for broiler chicks from young breeders to improve live body weight without affecting feed conversion ratio.

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