South African Journal of Animal Science 2020, 50 (No. 1)

Photoperiod effects on carcass traits, meat quality, and stress response in heart and lung of broilers

Y. Baykalir[1,*], U.G. Simsek[1], M. Erisir[2], O. Otlu[3], G. Gungoren[4], A. Gungoren[5] & S. Aslan[6]

[1]Department of Animal Science, Faculty of Veterinary Medicine, Firat University, Elazig 23200, Turkey
[2]Department of Biochemistry, Faculty of Veterinary Medicine, Firat University, Elazig 23200, Turkey
[3]Department of Genetic, Faculty of Veterinary Medicine, Firat University, Elazig 23200, Turkey
[4]Department of Animal Science, Faculty of Veterinary Medicine, Harran University, Sanliurfa 63100, Turkey
[5]Department of Food Hygiene and Technology, Faculty of Veterinary Medicine, Bingol University, Bingol 12100, Turkey
[6]Republic of Turkey, Ministry of Agriculture and Forestry, Veterinary Control Institute, Elazig 23100, Turkey

(Received 1 April 2019; Accepted 4 January 2020; First published online 17 March 2020)

Abstract

This study evaluated effects of photoperiod treatments on slaughter and carcass traits, meat quality, indicators of oxidative stress, and heat shock protein 70 (Hsp70) levels of lung and heart tissues in broilers. Five hundred Ross 308 broiler chicks were used. The treatments consisted of 23 hours of continuous light and one hour of darkness (23L1D), four hours of light followed by two hours of darkness (4L2D), eight hours of light and four hours of darkness (8L4D), and 16 hours of light and eight hours of darkness (16L8D). After 42 days, two birds from each replicate were slaughtered. Birds that had been subjected to 16L8D had lower slaughter, carcass, and breast weights than the other treatments. Significant correlations were observed for slaughter, carcass and breast weights and white stripe. At 10 min post mortem, the pH of the breast was the highest in 23L1D. Breasts from birds subjected to 23L1D and 16L8D had most fat and least protein, while white striping was not different among treatments. The 4L2D treatment resulted in the highest lung glutathione (GSH) concentration. Malondialdehyde (MDA) and GSH concentrations in the heart tissues of broilers from 8L4D and 4L2D were greater than those from 23L1D and 16:8. Glutathione peroxidase (GSH-Px) and superoxide dismutase concentrations were greatest for birds subjected to 16L8D. Heat shock protein 70 was lowest in lung and heart from birds subjected to 8L4D. Thus, shorter and more frequent periods of darkness can be recommended for welfare with little compromise in performance.

Keywords: carcass quality, heat shock protein 70, oxidative stress, white stripe
***Corresponding author: ybaykalir@firat.edu.tr

Introduction

Lighting is an important management technique in broiler production. Studies on lighting in broilers have focused on photoperiod, wavelength and light intensity (Deep *et al*., 2010; Mosa *et al*., 2015). These factors influence broiler performance and their welfare. Many companies implement continuous or near-continuous lighting regimens to maximize growth rate and feed intake (Olanrewaju *et al*., 2013). Low light intensities and increasing photoperiods also have positive effects on broiler performance and feed conversion (Lien *et al*., 2007). However, continuous lighting causes metabolic syndromes, which compromise the welfare of broiler chickens (Manser, 1996; Julian, 2000). In broiler flocks, sudden death syndrome (SDS), pulmonary hypertension syndrome (PHS), leg abnormalities, and abnormal eye development can all be reduced with intermittent lighting (Ononiwu *et al*., 1979; Li *et al*., 2000; Skrbic *et al*., 2015). The European Union (EU) has mandated that birds should be reared with lighting that follows a 24-hour rhythm and includes periods of darkness that last at least six hours in total, with at least one uninterrupted period of darkness of at least four hours (EU, 2007).

In fast-growing broiler chickens, PHS and SDS are problematic, and are affected by nutritional, management, environmental and genetic factors. Pulmonary hypertension syndrome occurs when lungs have insufficient pulmonary vascular capacity, and the right ventricle is forced to increase the pulmonary arterial pressure to propel the cardiac output throughout the lungs. Oxidative stress with depletion of

URL: http://www.sasas.co.za
ISSN 0375-1589 (print), ISSN 2221-4062 (online)
Publisher: South African Society for Animal Science
http://dx.doi.org/10.4314/sajas.v50i1.15

antioxidants and innate immunity also causes increases in pulmonary arterial pressure in broilers. Sudden death syndrome, also known as the flip-over disease, causes the birds to lie on their backs with outstretched wings and death occurs within 1 - 2 min. Economic losses from SDS in broilers flocks can be dramatic on occasion. The underlying causes of SDS include high-density diets, intensive feeding, and heat stress in broilers from three days old until slaughter age. However, it was reported that SDS is non-hereditary, unlike PHS (Saki & Matin 2011; Afolayan *et al*., 2016).

Antioxidant elements and heat shock proteins (Hsps) have a crucial role against cellular stress. Additionally, Hsps play an important role in maintaining protein homeostasis. Heat shock protein 70 is activated under stress conditions more than other Hsps. Intra-cytoplasmic Hsp70 might be increased in arterial smooth muscle cells when pulmonary hypertension occurs (Chen *et al*., 2018). Maximizing broiler growth rates and breast yield can lead to some alterations in their meat. The formation of white striping (WS) in the breast is a common manifestation, with an occurrence rate of around 12% being observed (Petracci *et al*., 2013). It is not of an infectious origin. However, muscle degeneration (hereditary muscular dystrophy) and myopathic changes have been seen in histological evaluations. Malnutrition, growth rate, sex, and slaughter weight were reported to be underlying causes for WS (Kuttappan *et al*., 2013). Such visually evident stripes on the breast surface have negative impacts on customer demands and marketing in the modern poultry industry (Petracci *et al*., 2014).

The effects of environmental factors on yields are well recognized. Lighting affects body composition and carcass quality (Downs *et al*., 2006). The poultry industry is pressured to reduce the incidence of fatty carcasses with poor low nutritious properties in accordance with customer demand (Kadim *et al*., 2005). Abdominal fat deposition has been reported to increase with prolonged periods of light (Newcombe *et al*., 1992). However, other researchers found no effects of photoperiod on abdominal fat and body composition (Charles *et al*., 1992; Renden *et al*., 1996). In addition, the effects of various photoperiod treatments on the fat and protein contents of broiler breast meat have not been documented. Thus, the current study aimed to evaluate carcass and meat quality characteristics, to estimate the correlations between carcass characteristics and WS, and to determine the oxidant/antioxidant status and Hsp70 levels of the lung and heart tissues under different photoperiod treatments in broiler.

Materials and Methods

A total of 500 male Ross 308 strain broiler chicks were used in this study. The chicks were raised at Firat University Agriculture and Livestock Research Centre (38° 35´ N, 39° 04´ E, altitude 1067 m). The 125 chicks were allocated randomly to four environmentally independent light-controlled rooms. Each room was subdivided into five replicates, which included 25 chicks. The stocking density was 12.5 birds/m^2 in all groups. The 24 hours constant light was implemented during seven days to all groups. At the end of seven days, experimental lighting was started. The first treatment consisted of 23 hours of light followed by 1 hour of dark. The second treatment had four repetitions of 4L2D in 24 hours. The third treatment had two repetitions of 8L4D in 24 hours and the fourth was 16L8D. Each room was lit with three white LED lamps (7 watts each) using with a light controller. The lighting was 1.75 W/m^2 and constant light density was maintained at eye level. Room temperature was applied as standard commercial practice with a gradual reduction from near hatching temperatures to 21 - 22 °C. The relative humidity was around 50 - 60% in all rooms. Wheat straw was used as the litter material. Standard commercial feeds based on corn and soybean meal (NRC, 1994) and drinking water were provided ad libitum. The feed ingredients and nutritional composition of the diets are shown in Table 1. At the end of 42 days, all birds in all five replicates were weighed and a total of 40 birds (two birds from each replicate) were selected for slaughtering based on the average bodyweight of those replicates. Animal use protocols were approved by Firat University Animal Researches Local Ethics Committee (FUHADYEK) (permission no: 17.13.2017/75). The feed was withdrawn eight hours before slaughter. After the slaughter process, every carcass was scalded at 60 °C for 90 seconds. The viscera, lungs, heads, and feet were removed manually, and carcasses were rinsed with water to remove blood, feathers, and other loose debris. Heart and lung tissues were reserved for chemical and western blot analyses and stored at -20°C until analysis. Hot carcass weight was then recorded. Breast fillets were removed manually, and left fillets were used to measure pH (Hanna, HI 99163, USA) within 10 min post mortem. Ether extract and crude protein analyses were also conducted on the left fillets. White striping on the fillets was scored visually by a qualified expert between 0 and 2 as 0 = no stripe, 1 = mild, and 2 = severe (Kuttappan *et al*., 2013).

An SER148 solvent extractor (Velp Scientifica, Italy) device was used for ether extract analysis according to AOAC 991.36 (2000). The samples were homogenized and wrapped in filter paper and were kept in 70 mL boiling solvent for 60 min. n-Hexane was used as a solvent. Immersion and washing were applied for 60 min. The samples were dried at 105 °C for 30 min and allowed to cool in a desiccator. The samples were weighed with an analytical scale (A&D, Japan), which has 0.001 g sensitivity.

Table 1 Feed ingredients and composition of diets fed to broilers reared under different photoperiod regimes

	Starter	Grower	Finisher
Feed ingredients			
Maize	54.10	45.70	54.50
Wheat	–	11.10	6.50
Vegetable oil	1.30	3.50	4.00
Soybean meal (48% crude protein)	30.10	25.10	24.50
Full fat soy	8.00	8.20	6.17
Meat and bone meal	3.00	3.27	–
Dicalcium phosphate	1.30	1.20	2.00
Ground limestone	0.50	0.30	0.70
Sodium bicarbonate	0.50	0.50	0.50
Salt	0.30	0.30	0.30
DL-Methionine	0.40	0.40	0.40
L-Lysine	0.10	0.05	0.05
Threonine	0.10	0.08	0.08
Vitamin mix[1]	0.20	0.20	0.20
Mineral mix[2]	0.10	0.10	0.10
Nutritional composition (%)			
Dry matter	90.60	90.10	90.89
Crude protein	23.40	22.00	19.70
Crude fibre	3.20	3.50	3.58
Ether extract	5.83	7.75	8.34
Ash	5.50	5.30	3.91
Calcium[3]	1.00	0.93	0.85
Available phosphorus[3]	0.51	0.51	0.44
Methionine[3]	0.69	0.66	0.59
Lysine[3]	1.44	1.27	1.11
Threonine[3]	0.97	0.88	0.81
Metabolizable energy (kcal/kg)[3]	3.01	3.18	3.23

[1] Vitamins (per kg): A: 12 000 000 IU; D3: 2 000 000 IU; E: 35 000 mg; K3: 4 000 mg; B1: 3 000 mg; B2: 7 000 mg; niacin: 20.000 mg; calcium D-pantothenate: 10 000 mg; B6: 5 000 mg; B12: 15 mg; Folic acid: 1 000 mg; D-biotin: 45 mg; C: 50 000 mg; choline chloride: 125.000 mg; canthaxanthin: 2 500 mg; ethyl ester of β-apo-8-carotenic acid: 500 mg
[2] Minerals (per kg): Manganese: 80 000 mg; Iron: 60 000 mg; Zinc: 60 000 mg; Copper: 5 000 mg; Cobalt: 200 mg; Iodine: 1 000 mg; Selenium: 150 mg
[3] Calculated values

The crude protein of the samples was analysed using the Dumas method with a Dumatherm protein analyser (Gerhardt, Germany). The dry matter content of the samples was determined. The samples were dried in an oven at 105 °C for 12 - 24 hours. They were shredded with a blender. One gram from each shredded sample was covered in airtight tinfoil and analysed by the device (AOAC 992.15, 2000).

Heart and lung tissues were homogenized in phosphate buffered saline. Malondialdehyde was measured with the thiobarbituric acid reactive substances (TBARS) method (Placer *et al*., 1966). Briefly, a pink complex formed MDA that it created with thiobarbituric acid (TBA) and the absorbance was read at 532 nm. Values of MDA were expressed as nmol/g tissue. Reduced GSH-Px (GSH) concentration of the tissues was determined according to the method of Chavan *et al*. (2005). Sulfhydryl groups in the tissue react with Ellman's reagent and form a yellow dye with maximum absorbance at 412 nm. Values were defined as µmol/g tissue.

Plasma glutathione peroxidase activity was assayed by the method of Matkovics *et al*. (1988). It was determined by using cumene hydroperoxide and reduced GSH as co-substrates, and the loss of GSH

following enzymatic reaction at 37 °C was measured spectrophotometrically (Schimadzu UV-1800, Japan) with Ellman's reagent at 412 nm. The enzyme activity was expressed as units per gr of protein (U/g protein). Superoxide dismutase (SOD) activity was measured using xanthine and xanthine oxidases to generate superoxide radicals, which react with nitroblue tetrazolium (NBT) (Sun *et al*., 1988). One unit of SOD activity was defined as the amount of enzyme required to cause inhibition of NBT. Superoxide dismutase activity was measured at 560 nm by the degree of inhibition of this reaction on a spectrophotometer and expressed as U/g protein.

Western blot procedure was carried out according to Baykalir & Simsek (2018). Samples of 0.5 g of lung and heart tissues were homogenized in 4.5 mL homogenization buffer (20 mM Tris pH: 7.4) at 17 000 rpm for 3 min with a blade-type homogenizer (Ultra Turrax T25, Germany) in an ice bath. Each sample was centrifuged at 31 000 g for 7 min at 4 °C (Hettich, USA). The supernatant was transferred to 1.5 mL tubes. Two aliquots of 1.5 mL were separated for protein quantification and electrophoresis. Total protein quantification was determined with the nanodrop spectrophotometer using 5 mg/ml bovine serum albumin as a standard. Loading sample buffer (Bio-Rad, 4x sample buffer, USA) was added to the electrophoresis samples and they were boiled at 95 °C for 5 min. Total protein was loaded as 30 µg/30 µl to 10% separating polyacrylamide gel for SDS-PAGE. Electrophoresis was carried out at a constant voltage of 150 V for 90 min (Bio-Rad, Mini Protean Tetra, USA). After SDS-PAGE, each gel was removed carefully and placed on 0.45 µm thick nitrocellulose membranes. Protein samples were transferred from gel to membrane under semi-dry conditions (Bio-Rad, Turbo Blotter, USA). The membranes were blocked with 5% bovine serum albumin diluted in Tris buffered saline + 0.01% Tween20 (TBST) solution for overnight under 4 °C. Then the membranes were incubated under 37 °C for two hours with mouse monoclonal anti-HSP70 antibody (Sigma, H5147) diluted with 1:1000 TBST. The membranes were washed four times (5 min) with TBST, then incubated with chicken anti-mouse IgG secondary antibody conjugated to horseradish peroxidase (Santa Cruz Biotech, sc-2954) with 1:5000 dilutions under 37 °C for 45 min. After the final wash with cold TBS solution, the bands were visualized with 3,3´-diaminobenzidine. For normalization of the bands, mouse monoclonal β-actin primary antibody (Santa Cruz Biotech, sc-47778) was used. The ImageJ (NIH image) image processing software program was used to evaluate relative densitometry of the bands.

The data were subjected to analysis of variance (one-way ANOVA) and correlation analysis with IBM SPSS 21 package program. Means of the groups were separated using Tukey's post-hoc method. Since the WS value was obtained by scoring, Spearman's correlation test was utilized to determine the relationship between WS and other carcass traits. The data were presented as mean and standard error of mean. The significant differences were accepted when $P \leq 0.05$ (Petrie & Watson, 2013).

Results and Discussion

The carcass traits and quality parameters are shown in Table 2. There were significant differences in slaughter, carcass, and breast weight. The 16L8D treatment resulted in a lower slaughter, carcass ($P < 0.01$), and breast ($P < 0.001$) weight than the other treatments. Significant correlations were also observed between slaughter, carcass and breast weight ($P < 0.001$), while these parameters did not correlate between pH, ether extract and crude protein ($P > 0.05$) (Table 3). The pH value within 10 min post mortem ($P < 0.001$) was the highest in 23L1D. The highest ether extract ($P < 0.01$) and the lowest crude protein ($P < 0.05$) were determined in 23L1D and 16L8D, while WS values ($P < 0.05$) were not different between groups.

Significant correlations were observed between slaughter ($P < 0.01$), carcass ($P < 0.001$), breast weight ($P < 0.01$), ether extract ($P < 0.05$), and WS (Table 3). In addition, ether extract was found to be correlated with crude protein ($P < 0.05$). Additionally, carcass traits of the groups are shown in Figure 1 and quality parameters are shown in Figure 2. Lighting has an important effect on feeding, drinking and other physical activities in poultry. Consumer demands of the carcass of a broiler chicken are focused on high breast weight and other valuable parts such as drumstick and thighs with a high weight (Faria *et al*., 2010). Slaughter weight was superior in 23L1D and 8L4D group. The 4L2D group and 16L8D group were similar to each other, with the 16L8D group being less than 23L1D and and 8D4L. The impact of photoperiod on slaughter performance in broiler chickens has had limited reports. It was suggested that without interruption, four hours of dark would have positive effects on the growth of broiler chickens and their carcass properties (Yang *et al*., 2015). In another study, intermittent lighting with 1 hour of light followed by 3 hours of dark (1L3D) did not influence hot dressing yield in broiler chickens (Onbasilar *et al*., 2007). Rahimi *et al*. (2005) found that the 1L3D schedule improved the feed conversion ratio and reduced abdominal fat in broilers, while bodyweight at 42 days was not influenced. However, significant correlations between slaughter, carcass and breast weights were observed in this study. Similar to the current study, pre-slaughter weight was highly correlated with dressing yield in Cobb 500 and Hubbard broiler strains (Pandurevic *et al*., 2014). Moreover, meat quality traits are influenced by slaughter weight. The pH of breast muscles was lower in light carcasses than heavy ones (Kadim *et al*., 2005; Yalcin *et al*., 2014). The pH of a meat is important for the

quality and shelf life of the carcass and is an indicator of the suitability of meat for various processing methods (Korkeala *et al*., 1986). It has been reported that the photoperiod regime of 14 hours of light and 10 hours of dark from 9 to 15 days, 16L8D from 16 to 22 days, 18 hours of light and 6 hours of dark from 23 to 29 days, and 20 hours of light and 4 hours of dark from 30 to 36 days did not affect pH value measured at the 15th minute post mortem or slaughter weight in Ross 308 broilers (Dereli Fidan *et al*., 2017). In this study, the higher pH value of the 23L1D group might be sourced from the higher carcass weight of the group. These variations between studies may depend on the implementation of different lighting programmes during the rearing phase.

Table 2 Effects of various photoperiod treatments on carcass characteristics and meat quality in broiler chickens

Treatment	Slaughter weight (kg)	Carcass weight (kg)	Breast weight (kg)	pH	Ether extract (%)	Crude protein (%)	WS (%)
23L1D	2.85 ± 0.05^{a}	2.19 ± 0.03^{a}	0.86 ± 0.01^{a}	6.35 ± 0.08^{a}	1.05 ± 0.16^{ab}	19.80 ± 1.07^{ab}	1.6 ± 0.16
8L4D	2.77 ± 0.06^{a}	2.20 ± 0.04^{a}	0.82 ± 0.02^{ab}	5.85 ± 0.09^{b}	0.52 ± 0.08^{b}	21.70 ± 0.29^{b}	1.1 ± 0.23
4L2D	2.69 ± 0.04^{ab}	2.08 ± 0.03^{ab}	0.76 ± 0.01^{bc}	5.75 ± 0.07^{b}	0.71 ± 0.14^{b}	21.92 ± 0.44^{b}	1.0 ± 0.25
16L8D	2.53 ± 0.08^{b}	2.00 ± 0.06^{b}	0.74 ± 0.02^{c}	5.91 ± 0.06^{b}	1.42 ± 0.23^{a}	19.10 ± 0.71^{a}	1.4 ± 0.27

WS: incidence of white striping; 23L1D: 23 hours light and 1 hour dark; 8L4D: 8 hours light and 4 hours dark; 4L2D: 4 hours light and 2 hours dark; 16L8D: 16 hours light and 8 hours dark

^{a,b,c} Within a column values with a common superscript do not differ (P >0.05)

Table 3 Pearson's correlation coefficients (r) among slaughter weight, carcass weight, breast weight and meat quality parameters in broiler chickens

	Carcass weight	Breast weight	pH	Ether extract	Crude protein	WS[1]
Slaughter weight	0,829***	0,766***	0,058	-0,125	0,100	0,449**
Carcass weight		0,879***	-0,007	-0,147	-0,073	0,458***
Breast weight			0,122	-0,065	-0,043	0,488**
pH				0,217	-0,096	0,111
Ether extract					-0,383*	0,366*
Crude protein						-0,043

*,**,*** Correlation different from 0 at the 0.05, 0.01, or 0.001 significance level, respectively
[1] Spearman correlation

Visual appearance is a unique factor when a consumer decides to buy any meat product. Nowadays, WS on breast fillets and thighs has negative effects on consumer purchases and causes a problem in the broiler meat market. In the current study, there were no differences in WS among the groups. However, there were correlations between slaughter, carcass, breast weight, ether extract and WS. Consistent with the current results, the heavier bodyweight and fat content were associated with WS (Kuttappan *et al*., 2012). Although the slaughter, carcass and breast weights were low in the 16L8D group, the amount of ether extract was high. In some studies, the rearing system, genotype and feed additives (especially fatty acids and probiotics) affected this feature (da Souza *et al*., 2011; Andi Murlina Tasse & Sandiah, 2015; Rubayet Bostami *et al*., 2017). According to the current study, increased fat accumulation, in parallel with the inactivity of birds that remain in the dark, may suggest an increase in ether extract levels. However, limit feeding of broiler chickens may lead to fat synthesis as feeding after prolonged hunger causes glucose and insulin

secretion. Under stress conditions, the blood glucose level is balanced by mobilizing from glycogen storage through the glucocorticoid corticosterone synthesis mechanism (Simsek *et al*., 2009). In addition, when chickens were raised under intermittent lighting, their energy expenditure was determined as low (Yang *et al*., 2015). Crude protein values were low in the 16L8D group. This finding could also be associated with prolonged fasting in darkness. Moreover, protein denaturation is higher in meats under pH 6 (Mir *et al*., 2017).

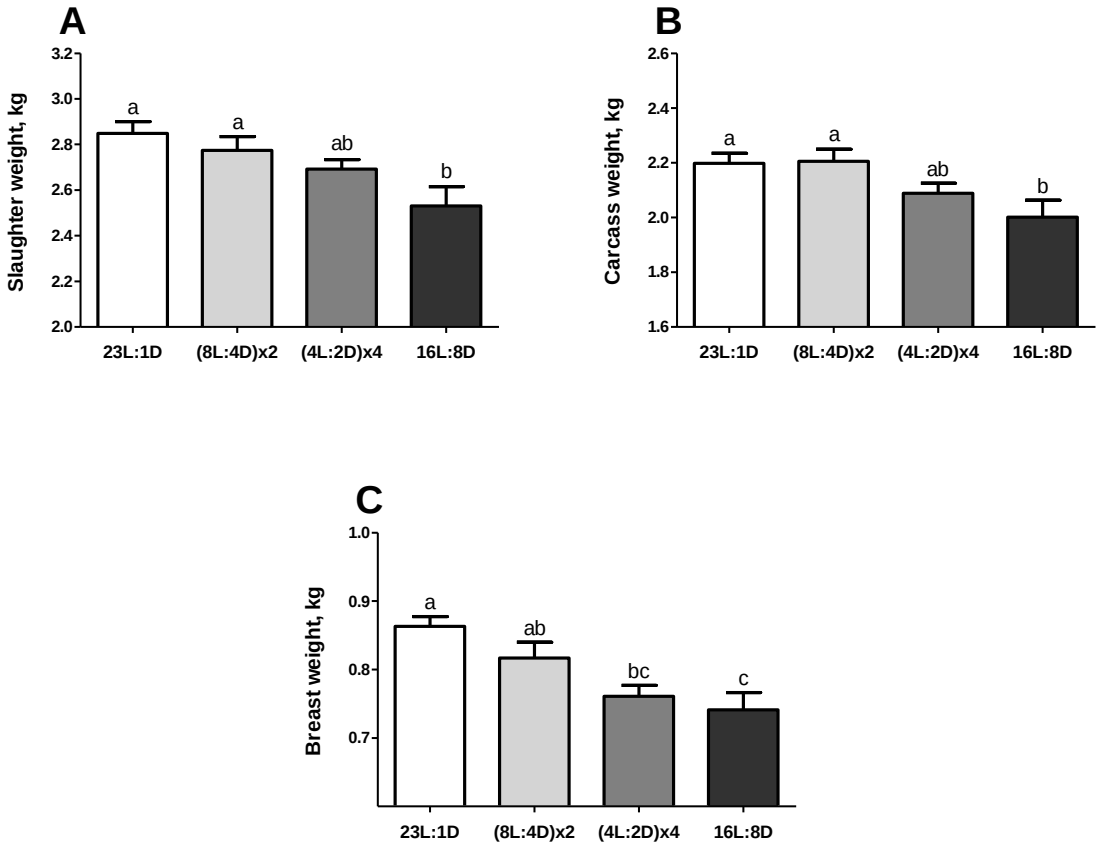

Figure 1 Slaughter, breast, and carcass weights of broiler chickens reared in different photoperiod regimes
[a,b,c] Bars with a common superscript do not differ at *P* =0.05
White bar (□): chicks were exposed to continuously 23 hours light (L) and 1 hour dark (D); light grey bar (▫): chicks were exposed to intermittently 4L2D, 4L2D, 4L2D and 4L2D; dark grey bar (▪): chicks were exposed to intermittently 8L4D and 8L4D; black bar (■): chicks were exposed to continuously 16L and 8D photoperiods

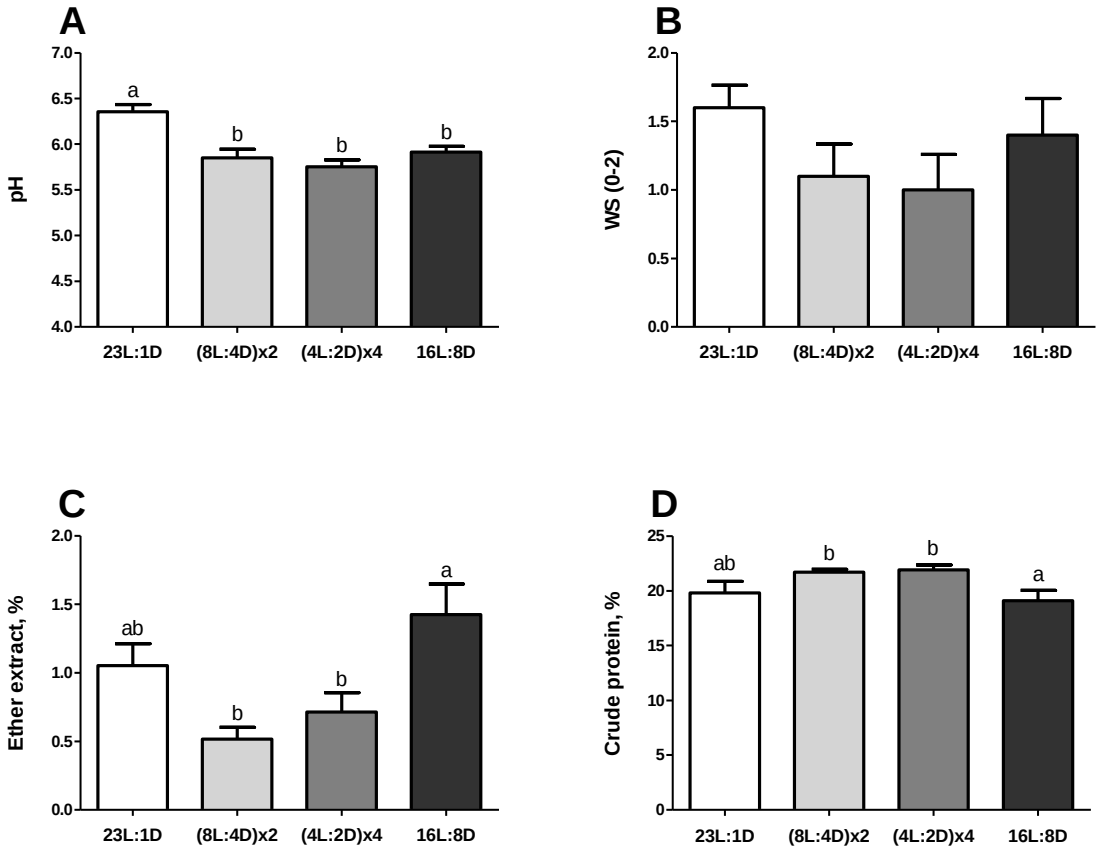

Figure 2 Percentages of ether extract and crude protein, pH and white striping of breast muscle from broiler chickens reared in different photoperiod regimes

[a, b] Bars with a common superscript do not differ at *P* =0.05

White bar (□): chicks were exposed to continuously 23 hours light (L) and 1 hour dark (D); light grey bar (□): chicks were exposed to intermittently 4L2D, 4L2D, 4L2D and 4L2D; dark grey bar (■): chicks were exposed to intermittently 8L4D and 8L4D; black bar (■): chicks were exposed to continuously 16L and 8D photoperiods

There were no differences between the groups, except for GSH in lung tissue (Table 4). Concentrations of MDA, GSH-Px, and SOD in the lungs were similar in all treatments (*P* >0.05) The 4L:4D treatment resulted in a higher GSH concentration than the other treatments (*P* <0.001). The MDA and GSH concentrations in heart tissues of broilers from 8L4D and 4L2D treatments were higher than those of 23L1D and 16L8D birds. Plasma glutathione peroxidase and SOD concentrations were highest in the 16L8D treatment (*P* <0.001) (Table 4). Pulmonary hypertension syndrome is also known as ascites syndrome and causes severe problems in fast-growing broiler chickens. Lung and heart functions fall behind this rapid growth rate and therefore circulatory disorders occur. Besides the fast growth rate, genetic and nutritional factors, high altitude and *Enterococcus faecalis* have been reported to cause PHS (Tankson *et al*., 2001; Khajali *et al*., 2016; Rodriguez-Ortega *et al*., 2017). Pulmonary hypertension syndrome is also reported to be associated with oxidative stress. Antioxidants on lipid peroxidation had beneficial effects on PHS in broilers (Bottje *et al*., 1995). On the other hand, SDS risk can be reduced with antioxidant feed additives in broiler flocks (Hassanzadeh *et al*., 2014). In this study, only reduced GSH was different in the lungs among the groups. The heart was the organ that was most affected by oxidative stress. In another study, intermittent lighting did not influence serum antioxidant enzymes. It was stated that this was because of the fluctuation of melatonin synthesis (Mosleh *et al*., 2016). Melatonin has also a scavenger effect on free radicals and is a wide-spectrum antioxidant. Although melatonin synthesis increases in darkness, in a bright environment decreases in melatonin may activate GSH-Px and SOD in broiler chickens (Albarrán *et al*., 2001). Indeed, the concentrations of GSH-Px and SOD in the 16L8D group, especially in the heart, were high. Intermittent lighting may also cause stress in chickens because it may stimulate the formation of cellular free radicals from sleeping disorders in broiler chickens. Thus, MDA formation can be expected to be high in the

intermittent lighting groups. Partial pressure of oxygen reduces at high altitude and causes hypoxia. As a result, the formation of reactive oxygen species was determined in the heart and liver of broilers (Diaz-Cruz *et al*., 1996). In broiler farming, feeding is applied ad libitum to increase the growth rate. On the other hand, ad libitum feeding decreases antioxidant activity in the lungs, hearts, and livers of broilers (Rodriguez-Ortega *et al*., 2014). In this study, in the 16L8D group levels of MDA and antioxidant parameters were high in the heart. This might be because antioxidant activity was trying to reduce the high MDA levels. In addition, MDA formation could be stimulated because the broilers could not reach to feed during the long dark period (Rodriguez-Ortega *et al*., 2017; Baykalir & Simsek, 2018).

The relative density of the Hsp70 bands in the lungs was higher in 16L8D than other groups (P<0.01). Heat shock protein 70 of the heart tissues from the 4L:4D treatment had a higher relative density than other treatments. Heat shock protein 70 levels from the 8L4D treatment had the lowest in both tissues (P<0.001) (Figure 3). Heat shock proteins play a vital role in obtaining an active form of proteins and in protein homeostasis. A protein that is synthesized must undergo some folds to perform its function. Newly synthesized protein can be susceptible to inaccurate folding, especially in stressful conditions. At the same time, Hsps have a protective effect in eliminating the negative effects of stress as a defence system. They are classified according to their molecular weights between 10 and 150 kDa. The Hsp70 family are most activated under stress conditions. Studies on Hsp70 focused on heat/cold stress and rearing systems in poultry farming (Givisiez *et al*., 2000; Gu *et al*., 2012; Baykalir & Simsek, 2018). Furthermore, Hsp70 was accepted as a marker of heat stress at cellular level in buffalo heifers and zebu cattle (Hansen, 2004; Patir & Upadhyay, 2010). However, there was no literature on alterations in Hsp70 under different photoperiod treatments in broilers. In this study, lung Hsp70 level was high in the 16L8D group and the heart Hsp70 level was the lowest in 8L4D group, and highest in 4L2D group. Furthermore, in this study, the Hsp70 level in the 16L8D treatment was high in the lung tissue, but not so high in the heart. The high antioxidant activity may have functioned sufficiently in the heart to not need to increase the Hsp70 level in the 16L8D birds. In addition, cardiac tissue has been reported to be more sensitive than pulmonary tissue to oxidative stress (Damiani *et al*., 2012). In the 4L2D group, compared with other groups, a bright environment immediately after a short dark period might cause stress in broilers and increase Hsp70 levels. This may be associated with a sleeping disorder.

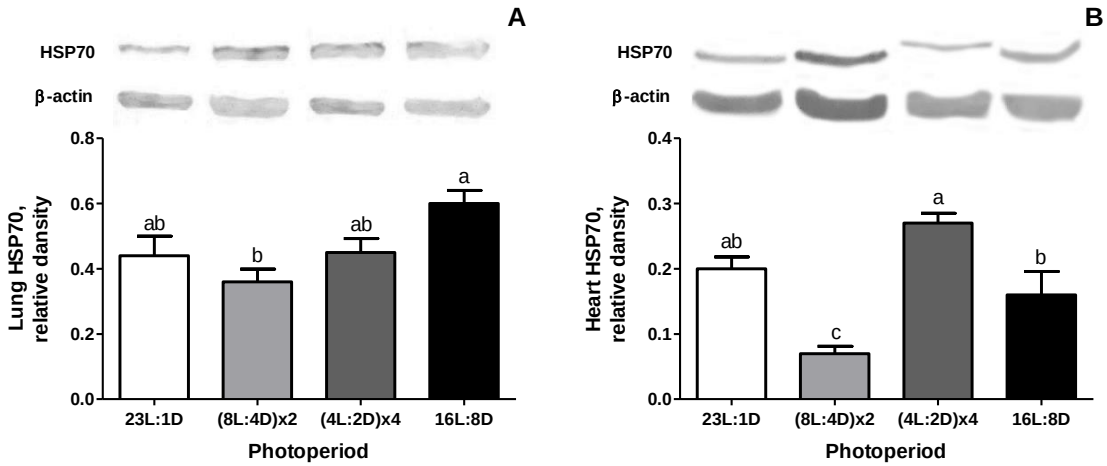

Figure 3 Effects of various photoperiod treatments on lung and heart heat shock protein 70 levels in broiler chickens

Each bar is presented as mean and standard error. Western blot analysis was performed with the inclusion of actin to ensure equal protein loading. The relative density of the bands was calculated based on the actin. Blots were repeated at least three times

[a, b, c] Values within the bars with different superscripts are different (Turkey's post-hoc test, *P* <0.05)

1 **Table 4** Effects of various photoperiod treatments on some oxidative stress parameters of lung and heart tissues in broiler chickens
2

Photoperiod	Lung				Heart			
	MDA (nmol/g tissue)	GSH (µmol/g tissue)	GSH-Px (U/g protein)	SOD (U/g protein)	MDA (nmol/g tissue)	GSH (µmol/g tissue)	GSH-Px (U/g protein)	SOD (U/g protein)
23L1D	13.18 ± 1.55	0.77 ± 0.07^{b}	15.83 ± 1.46	193.51 ± 8.35	1.26 ± 0.31^{b}	0.56 ± 0.04^{b}	29.10 ± 1.48^{a}	276.61 ± 14.88^{a}
(8L4D)x2	13.78 ± 2.21	0.86 ± 0.10^{ab}	17.48 ± 1.40	201.15 ± 9.98	2.10 ± 0.29^{ab}	0.81 ± 0.07^{a}	32.57 ± 1.53^{a}	277.14 ± 14.14^{a}
(4L2D)x4	12.94 ± 0.98	1.15 ± 0.13^{a}	14.62 ± 1.10	192.82 ± 6.94	2.56 ± 0.22^{ab}	0.74 ± 0.06^{ab}	33.14 ± 1.47^{a}	295.84 ± 10.89^{a}
16L8D	13.99 ± 1.60	0.26 ± 0.03^{c}	14.76 ± 1.83	182.29 ± 9.29	1.99 ± 0.20^{a}	0.65 ± 0.03^{ab}	45.90 ± 2,32^{b}	417.68 ± 13.43^{b}

^{a,b,c} Values with a common superscript do not differ (P <0.05); MDA: malondialdehyde; GSH: glutathione; GSH-Px: plasma glutathione peroxidase; 23L1D: 23 hours light and 1 hour dark; 8L4D: 8 hours light and 4 hours dark; 4L2D: 4 hours light and 2 hours dark; 16L8D: 16 hours light and 8 hours dark

Conclusion

The 8L4D treatment had a positive impact on broilers in terms of both carcass traits and stress. The carcass characteristics of 8L4D were found to be proximate and satisfactory to near-continuous lighting that is applied in modern broiler farming. Intermittent lighting was found to be better than the longer dark treatment that is recommended for welfare.

ORCID Numbers

Yasin Baykalir: 0000-0002-9248-6065; Ulku Gulcihan Simsek: 0000-0003-2871-3005; Mine Erisir: 0000-0001-6209-4792; Onder Otlu: 0000-0001-5958-7609; Gulsah Gungoren: 0000-0002-0360-7735; Alper Gungoren: 0000-0001-7818-1372; Sultan Aslan: 0000-0001-8480-1515

Acknowledgements

The authors would like to thank Firat University Department of Scientific Research Projects for providing financial support (FUBAP, Grant VF.17.17), Elazig, Turkey.

Authors' Contributions

UGS was in charge of conception and design of study. All authors participated in the acquisition of data. YB drafted and wrote the manuscript. Critical reviews/revisions and interpreting of data were made by UGS and YB.

Conflict of Interest Declaration

The authors certify that there is no conflict of interest with any financial organization regarding the material discussed in the manuscript.

References

Afolayan, M., Abeke, F.O. & Atanda, A., 2016. Ascites versus sudden death syndrome (SDS) in broiler chickens: A review. J. Anim. Prod. Res. 28, 76-87.

Albarrán, M.T., López-Burillo, S., Pablos, M.I., Reiter, R.J. & Agapito, M.T., 2001. Endogenous rhythms of melatonin, total antioxidant status and superoxide dismutase activity in several tissues of chick and their inhibition by light. J. Pineal Res. 30, 227-233.

Andi Murlina Tasse, F. & Sandiah, N., 2015. Chemical composition of broiler chicken breast fed ration with supplementation of protected fatty acid. In: Proceedings of International Seminar "Improving Tropical Animal Production for Food Security" 3-5 November 2015, Southeast Sulawesi, Indonesia, 154-157.

AOAC International, 2000. Official methods of analysis. 17th edition. Fat (crude) in meat and meat products. Official Method 991.36. AOAC, Gaithersburg, MD.

AOAC International, 2000. Official methods of analysis. 17th edition. Fat (crude) in meat and meat products. Official Method 992.15. AOAC, Gaithersburg, MD.

Baykalir, Y. & Simsek, U.G., 2018. Impact of different rearing systems and age on Bovans White layers' performance, egg quality traits and synthesis of heat shock protein 70 kDa. Ann. Anim. Sci. 18, 1045-1060.

Bottje, W., Enkvetchakul, B. & More, R., 1995. Effect of α-tocopherol on antioxidants, lipid peroxidation, and the incidence of pulmonary hypertension syndrome (ascites) in broilers. Poult. Sci. 74, 1356-1369.

Charles, R.G., Robinson, F.E., Hardin, R.T., Yu, M.W., Feddes, J. & Classen, H.L., 1992 Growth, body composition, and plasma androgen concentration of male broiler chickens subjected to different regimens of photoperiod and light intensity. Poult. Sci. 71, 1595-1605.

Chavan, S., Sava, L., Saxena, V., Pillai, S., Sontakke, A. & Ingole, D. 2005. Reduced glutathione: Importance of specimen collection. Indian J. Clin. Biochem. 20, 150-152.

Chen, F., Wang, H., Yan, J., Lai, J., Cai, S., Yuan, L. & Zheng, S., 2018. Grape seed proanthocyanidin reverses pulmonary vascular remodeling in monocrotaline-induced pulmonary arterial hypertension by downregulating HSP70. Biomed. Pharmacother. 101, 123-128.

da Souza, X.R., Faria, P.B. & Bressan, M.C., 2011. Proximate composition and meat quality of broilers reared under different production systems. Braz. J. Poultry Sci. 13, 15-20.

Damiani, R.M., Piva, M.O., Petry, M.R., Saldiva, P.H.N., de Oliveira, A.T.D. & Rhoden, R.C., 2012. Is cardiac tissue more susceptible than lung to oxidative effects induced by chronic nasotropic instillation of residual oil fly ash (ROFA)? Toxicol. Mech. Meth. 22, 533-539.

Deep, A., Schwean-Lardner, K., Crowe, T.G., Fancher, B.I. & Classen HL., 2010. Effect of light intensity on broiler production, processing characteristics, and welfare. Poult. Sci. 89, 2326-2333.

Dereli Fidan, E., Nazligul, A., Turkyilmaz, M.K., Unubol Aypak, S., Kilimci, F.S., Karaarslan, S. & Kaya, M., 2017. Effect of photoperiod length and light intensity on some welfare criteria, carcass, and meat quality characteristics in broilers. R. Bras. Zootec. 46, 202-210.

Diaz-Cruz, A., Nava, C., Villanueva, R., Serret, M., Guinzberg, R. & Pina, E., 1996. Hepatic and cardiac oxidative stress and other metabolic changes in broilers with the ascites syndrome. Poult. Sci. 75, 900-903.

Downs, K.M., Lien, R.J., Hess, J.B., Bilgili, S.F. & Dozier, W.A., 2006. The effects of photoperiod length, light intensity, and feed energy on growth responses and meat yield of broilers. J. Appl. Poult. Res. 15, 406-416.

EU, 2007. Council directive 2007/43/EC laying down minimum rules for the protection of chickens kept for meat production. Official Journal of the European Union 182, 19-28.

Faria, P.B., Bressan, M.C., de Souza X.R., Rossato, L.V., Botega, L.M.G. & da Gama, L.T., 2010. Carcass and parts yield of broilers reared under a semi-extensive system. Braz. J. Poultry Sci. 12, 153-159.
Givisiez, P.E.N., da Silva, M.M., Mazzi, C.M., Ferro, M.I.T., Ferro, J.A., Gonzales, E & Macari, M., 2000. Heat or cold chronic stress affects organ weights and Hsp70 levels in chicken embryos. Can. J. Anim. Sci. 81, 83-87.
Gu, X.H., Hao, Y. & Wang, X.L., 2012. Overexpression of heat shock protein 70 and its relationship to intestine under acute heat stress in broilers: 2. Intestinal oxidative stress. Poult. Sci. 91, 790-799.
Hansen, P.J., 2004. Physiological and cellular adaptations of zebu cattle to thermal stress. Anim. Reprod. Sci. 82-83, 349-360.
Hassanzadeh, M., Buyse, J., Toloei, T. & Decuypere, E., 2014. Ascites syndrome in broiler chickens: A review on the aspect of endogenous and exogenous factors interactions. J. Poult. Sci. 51, 229-241.
Julian, R.J., 2000. Physiological, management and environmental triggers of the ascites syndrome: A review. Avian Pathol. 29, 519-527.
Kadim, I.T., Mahgoub, O., Al-Marzooqi, W. & Annamalai, K., 2005. Prediction of crude protein, extractable fat, calcium and phosphorus contents of broiler chicken carcasses using near-infrared reflectance spectroscopy. Asian-Aust. J. Anim. Sci. 18, 1036-1040.
Khajali, F. & Wideman, R.F., 2016. Nutritional approaches to ameliorate pulmonary hypertension in broiler chickens. J. Anim. Physiol. Anim. Nutr. 100, 3-14.
Korkeala, H., Maki-Petays, O., Alanko, T. & Sorvettula O., 1986. Determination of pH in meat. Meat Sci. 18, 121-132.
Kuttappan, V.A., Brewer, V.B., Waldroup, P.W. & Owens, C.M., 2012. Influence of growth rate on the occurrence of white striping in broiler breast fillets. Poult. Sci. 91, 2677-2685.
Kuttappan, V.A., Shivaprasad, H.L., Shaw, D.P., Valentine, B.A., Hargis, B.M., Clark, F.D., McKee, S.R. & Owens, C.M., 2013. Pathological changes associated with white striping in broiler breast muscles. Poult. Sci. 92, 331-338.
Li, T., Howland, H.C. & Troilo, D., 2000. Diurnal illumination patterns affect the development of the chick eye. Vision Res. 40, 2387-2393.
Lien, R.J., Hess, J.B., McKee, S.R., Bilgili, S.F. & Townsend, J.C., 2007. Impact of light intensity and photoperiod on live performance, heterophil to lymphocyte ratio, and processing yields of broilers. Poult. Sci. 86, 1287-1293.
Manser, C.E., 1996. Effects of lighting on the welfare of domestic poultry: A review. Anim. Welf. 5, 341-360.
Matkovics, B., Szabo, I. & Varga, I.S., 1988. Determination of enzyme activities in lipid peroxidation and glutathione pathways. Laboratoriumi Diagnosztika 15, 248-249.
Mir, N.A., Rafiq, A., Kumar, F., Singh, V. & Shukla, V., 2017. Determinants of broiler chicken meat quality and factors affecting them: a review. J. Food Sci. Technol. 54, 2997-3009.
Mosa, R.K., Rabia, J., Abbas, R.J. & Abu Tabeekh, M.A.S., 2015. An investigation on light color and stocking density on some productive performance of broilers. Bas. J. Vet. Res. 14, 176-186.
Mosleh, N., Amirmoezzi, F. & Nazifi, S., 2016. Intermittent photoperiod schedule does not influence brain and serum melatonin and selected serum antioxidant enzymes activity in broiler chickens. Poult. Sci. J. 4, 81-87.
Newcombe, M., Cartwright, A.L. & Harter-Dennis, J.M., 1992. The effect of increasing photoperiod and food restriction in sexed broiler-type birds. I. Growth and abdominal fat cellularity. Br. Poult. Sci. 33, 415-425.
NRC, 1994. Nutrient requirements of poultry. 9th revised edition. The National Academies Press, Washington, DC, USA.
Olanrewaju, H.A., Purswell, J.L., Collier, S.D. & Branton, S.L., 2013. Interactive effects of photoperiod and light intensity on blood physiological and biochemical reactions of broilers grown to heavy weights. Poult. Sci. 92, 1029-1039.
Onbasilar, E.E., Erol, H., Cantekin, Z. & Kaya, U., 2007. Influence of intermittent lighting on broiler performance, incidence of tibial dyschondroplasia, tonic immobility, some blood parameters and antibody production. Asian-Aust. J. Anim. Sci. 20, 550-555.
Ononiwu, J.C., Thomson, R.G., Carlson, H.C. & Julian, R.J., 1979. Pathological studies of “sudden death syndrome” in broiler chickens. Can. Vet. J. 20, 70-73.
Pandurevic, T. & Lalovic, M., 2014. Correlation between body weight before slaughter and slaughter yields broiler carcasses of different lines. Acta Agric. Serb. 19, 151-157.
Patir, H. & Upadhyay, R.C., 2010. Purification, characterization and expression kinetics of heat shock protein 70 from Bubalus bubalis. Res. Vet. Sci. 88, 258-262
Petracci, M., Mudalal, S., Babini, E. & Cavani C., 2014. Effect of white striping on chemical composition and nutritional value of chicken breast meat. Ital. J. Anim. Sci. 13, 179-183.
Petracci, M., Mudalal, S., Bonfiglio, A. & Cavani, C., 2013. Occurrence of white striping under commercial conditions and its impact on breast meat quality in broiler chickens. Poult. Sci. 92, 1670-1675.
Petrie, A. & Watson, P., 2013. Statistics for veterinary and animal science. 3rd edition. Wiley-Blackwell, Hoboken, NJ, USA.
Placer, Z.A., Cushman, L.L. & Johson, B.C., 1966. Estimation of product of lipid peroxidation (malonyl dialdehyde) in biochemical systems. Anal. Biochem. 16, 359-364.
Rahimi, G., Rezaei, M., Hafezian, H. & Saiyahzadeh, H., 2005. The effect of intermittent lighting schedule on broiler performance. Int. J. Poult. Sci. 4:396-398.
Renden, J.A., Jr Moran, E.T. & Kincaid, S.A., 1996. Lighting programs for broilers that reduce leg problems without loss of performance or yield. Poult. Sci. 75, 1345-1350.
Rodríguez-Ortega L.T., Juárez-Juárez G., Pro-Martínez A., Sosa-Montes E., Bautista-Ortega J., González-Cerón F., Vargas-Galicia A.J., Chan-Díaz D., Moreno-Medina D., Gallegos-Sánchez J. & Rodríguez-Ortega A., 2017. Lipid peroxidation in the plasma, lungs, heart and liver of broilers fed a grape seed extract and raised at 2278 m of altitude. Braz. J. Poultry Sci. 19, 465-470.

Rodríguez-Ortega, L.T., Sosa-Montes, E., Pro-Martínez A., Ruiz-Feria, C.A., Bautista-Ortega, J., Vargas-Galicia AJ., 2014. Free access to feed reduces the antioxidative capacity in broilers raised at high altitudes. Poult. Sci. 93(E-Supp. 1), 77.

Rubayet Bostami, A.B.M., Mun, H.S. & Yang, C.J., 2017. Breast and thigh meat chemical composition and fatty acid profile in broilers fed diet with dietary fat sources. J. Food Process Technol. 8, 1-7.

Saki, A.A. & Matin, H.R.H., 2011. Does nutrition help to alleviate sudden death syndrome in broiler chicken? An overview. Glob. Vet. 6, 262-268.

Simsek, U.G., Cerci, I.H., Dalkilic, B., Yilmaz, O. & Ciftci M., 2009. Impact of stocking density and feeding regimen on broilers: Chicken meat composition, fatty acids, and serum cholesterol levels. J. Appl. Poultry Res. 18, 514-520.

Skrbic, Z., Pavlovski, Z., Lukic, M. & Petricevic, V., 2015. Incidence of footpad dermatitis and hock burns in broilers as affected by genotype, lighting program and litter type. Ann. Anim. Sci. 15, 433-445.

Sun, Y., Oberley, L.W. & Li, Y., 1988. A simple method for clinical assay of superoxide dismutase. Clin. Chem. 34, 497-500.

Tankson, J.D., Thaxton, J.P. & Vizzier-Thaxton, Y., 2001. Pulmonary hypertension syndrome in broilers caused by *Enterococcus faecalis.* Infect. Immun. 69, 6318-6322.

Yalcin, S., Guler, H.C., Yasa, I., Turgay Izzetoglu, G. & Ozkan, S., 2014. Effect of breeder age and slaughter weight on meat quality traits of broiler breast and leg meats. Europ. Poult. Sci. 78, 1-10.

Yang, H., Xing, H., Wang, Z., Xia, J., Wan, Y., Hou, B. & Zhang, J., 2015. Effects of intermittent lighting on broiler growth performance, slaughter performance, serum biochemical parameters and tibia parameters. Ital. J. Anim. Sci. 14, 684-689.