Intermittent lighting regime as a tool to enhance egg production and eggshell thickness in Rhode Island Red laying hens

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ABSTRACT Influences of intermittent light regime as a tool to enhance egg production, egg quality, and blood parameters of laying hens were investigated. A total of 270 hens of Rhode Island Red (during 20 to 36 wk of age) were used to investigate the effects of intermittent light regime in completely randomized design. The birds were divided into 3 equal groups (6 replicates of 15 birds each) and housed in floor pens. The first group was served as non-treated control (C) and was exposed to continuous and constant light for 16 h light/day throughout the experimental period. Whereas, birds of the other groups were exposed to intermittent lights for 20 min/h + 40 min of constant light (T1; FLASH20) and 40 min/h + 20 min of constant light (T2; FLASH40) during the 16 h of light period. Hens of T1 group showed significantly ($P \leq 0.05$) the highest concentration of total antioxidant capacity and the lowest one of malondialdehyde in comparison with the other groups. Hens of T1 group had significantly ($P \leq 0.05$) the greatest egg laying rate and egg mass in comparison with the other counterparts. Feed consumption was similar in the groups under study. Hens exposed to FLASH20 had the lowest ($P \leq 0.05$) FCR when compared with the other treatments. Eggs produced from hens exposed to FLASH20 had the highest value of shell thickness followed by the control and then that of those exposed to FLASH40. There were insignificant differences among the treatments in body weight of hens and all of other egg quality and egg problem traits. In conclusion, intermittent light regime of 20 min/h was the most efficient in comparison with the other ones. Finally, intermittent light regime of 20 min/h during laying period (during 20 to 36 wk of age) is highly recommended.

Key words: blood parameters, egg laying rate, egg quality, laying hens, light

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INTRODUCTION

In intensive egg production, one of the main technological elements in determining the productivity is lighting and its properties. Lighting influences several physiological processes (including stimulation of internal organs and initiation of hormone release, and various metabolic steps that facilitate feeding and digestion), egg production rate and egg mass, and feed efficiency in laying hens (Durmuş and Kalebaşı, 2009; Ma et al., 2013; Geng et al., 2014; Farghly and Makled, 2015; Molino et al., 2015). The pattern and duration of the lighting regime allows the hen to establish a circadian rhythm (Dawson et al., 2001).

Poultry species receive light through the pineal gland, the hypothalamus, and its photoreceptors that have the ability to absorb light, penetrating the skull (Li and Howland, 2003; Thiele, 2009; Jácome et al., 2014). Therefore, the pineal gland appears to translate environmental cues into melatonin excretion that are necessary for daily regulation of cardiopulmonary, reproductive, excretory, thermoregulatory, behavioral, and immune systems (Pang et al., 1996; Abbas et al., 2007; Navara and Nelson, 2007). Keeping birds under long darkness periods results in fewer health-related problems than those kept in continuous or constant light (Moore and Siopes, 2000; Farghly and Makled, 2015).

Intermittent lighting programs were used to improve feed efficiency and egg production of laying hens. Due to the reduction in the physical activity during darkness, increasing resting and energy expenditure of activity

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is considerable (Rahimi et al., 2005; Ma et al., 2013; Farghly, 2014; Farghly and Makled, 2015; Yuri et al., 2016). Intermittent lighting regimes save about 5% of feed consumption (Morris, 2004). Light flashes can be widely used to enhance production efficiency and feed conversion ratio (**FCR**), and as a way to save costs via reducing electricity exhaustion (Farghly and Makled, 2015; Farghly et al., 2016).

Subjective day is the period during which the bird is awake and physiologically active, even if it is in the dark. This allows the use of intermittent lighting programs for laying hens, which are programs that include more than one period of light (photophase) and one period of dark (scotophase) within a 24-h cycle (Gewehr and Freitas, 2007; Gewehr et al., 2010). One of the interesting sides of the management of laying hens is that they do not need continuous periods of illumination. This process is called "subjective day," which indicates that laying hens neglect periods of dark between the 14 and 16 h of light stimulation. One of the intermittent lighting programs, that is the so-called biomittent lighting, consists of fractioning the time of alternate light and dark cycles as 25%L: 75%D (Jácome et al., 2014). Therefore, the objective of this experiment was to study the impact of using light flashes on blood parameters and some antioxidant markers, egg production rate, and some of egg quality traits of Rhode Island Red hens.

MATERIALS AND METHODS

Experimental Design, Birds, Diets, and Husbandry

The present study was carried out at the Research Poultry Farm, Poultry Production Department, Faculty of Agriculture, Asyut University. The experimental procedures used in the current work were approved by Ethics Committee for Animal Experimentation of Poultry Production Department, Faculty of Agriculture, Asyut University, Egypt. A total of 270 20-wk-old hens were divided into 3 equal groups (6 replicates of 15 birds each) and housed in floor pens (18 pens of 1 × 2 m) in an open-sided house. The first group was served as non-treated control (C) and was exposed to continuous and constant light for 16 h light/day throughout the experimental period. Whereas, birds of the other groups were exposed to intermittent lights for 20 min/h + 40 min of constant light (T1; FLASH20) and 40 min/h + 20 min of constant light (T2; FLASH40) during the 16 h of light period. Each pen was equipped with two 25-W light incandescent bulbs placed 1.50 m from the floor to provide 15 to 20 lux of light at the floor level. All sources of natural light were covered with heavy cotton black curtains and blackout plastic curtains that completely prevent any source of natural light. Light flashes were composed of 20 or 40 flashes/min provided by using incandescent bulbs. Light flashes were defined as flashing lights with suitable intensity at bird level, which were generated by flasher apparatus that contained timer and dimmer to justify the flashed lighting period and intensity. Feed and clean water were available ad libitum, and all the other conditions were the same during the experimental period (20 to 36 wk of age). The composition and calculated analysis of the experimental diet are shown in Table 1. The experimental birds were maintained under temperature conditions of 24 to 26°C during the experimental period.

Table 1. Composition and calculated analysis of the experimental diet.

Ingredients	(g/k; as –fed basis)
Yellow corn	695
Soybean meal (44%)	150
Layer concentrate[1]	80
Salt	1.00
Minerals	–
Premix	-
Bone meal	4.00
Limestone	70
Total	1,000
Calculated analysis[2]	
Crude protein	174
ME MJ/kg diet	12.00
Calcium	31.00
Available phosphorus	3.70

[1]Layer concentrate.
[2]Calculated according to NRC (1994).

Data Collection and Calculations

At the end of the experiment, blood samples were collected from the wing vein in heparinized tubes. Blood samples were centrifuged at 3,000 rpm for 15 min, and plasma obtained was stored at −20°C until analysis. Plasma total protein, albumin, total cholesterol, and transaminase enzymes activities (Aspartate aminotransferase (**AST**) and alanine aminotransferase (**ALT**)) were determined colorimetrically using available commercial kits purchased from Spectrum Diagnostic Company (Cairo, Egypt). Globulin values were obtained by subtracting the values of albumin from the corresponding values of total protein. Blood plasma concentrations of triiodothyronine (T_3) were determined in blood plasma according to the method of Britton et al. (1975). Total antioxidant capacity (**T-AOC**) and malondialdehyde (**MDA**) were measured according tothe method described by Koracevic et al. (2001).

Egg weight, egg number, and hen-day egg production (**HDP**) were counted and recorded from 24 to 36 wk of age. During the period from 24 to 36 wk of the experiment, 36 fresh-laid eggs were taken, every 4 wk, from each group to measure egg quality characteristics. FCR (g feed/g egg) was calculated biweekly. Egg weight was recorded to the nearest 0.1 g on the same day of collection using electronic scale. The length and width of each egg were determined using a sliding caliper, and their egg shape index = (width of egg/length of egg) × 100 was calculated. Shell thickness of the dried shell (without membranes) was measured using shell

thickness apparatus (millimeters). The heights of thick albumen and yolk were measured using a micrometer. Haugh unit values were calculated for each egg using the formula: Haugh unit = 100 log (H- 1.7 × W 0.37 + 7.6), where H = the observed height of the albumen in millimeters and W = weight of egg (g). The yolk index was calculated by dividing (yolk's height/yolk's diameter) × 100. In addition, shells with membranes were dried and weighed to the nearest 0.01 g. Egg problems percent (floor, cracks, and dirty eggs) were observed and recorded daily for each pen.

Statistical Analysis

The experimental design used in the present work was completely randomized. Data collected were subjected to analysis of variance (**ANOVA**) by applying the general linear model procedure of SAS software (SAS, 2008). All means were tested for significant differences using Duncan's multiple range procedure (Duncan, 1955). The following statistical model was used for ANOVA:

$$Y_{ij} = \mu + S_i + e_{ij},$$

where Y_{ij} = an observation, μ = the overall mean, S_i = treatment effect, and e_{ij} = experimental random error. The replicate was the experimental unit in the present work.

RESULTS

Blood Parameters and Antioxidant Markers

Results of blood parameters and antioxidant markers as affected by flash light are presented in Table 2. There were non-significant differences in blood parameters (these values were in the normal range) and T_3 due to the intermittent light among the treated groups. The same group of intermittent light had significantly the highest concentration of T-AOC ($P = 0.0221$) and the lowest one of MDA ($P = 0.0435$) in comparison with the other groups. Results of T2 group that exposed to FLASH40 were intermediate.

Productive Traits

Data found in Table 3 clearly indicate the effects of lighting program on the productive traits. Body weight (**BW**) of T1 group was insignificantly the heaviest as compared with the other groups. Feed consumption was similar in the groups under study. Hens exposed to FLASH20 had significantly ($P = 0.0438$) the lowest FCR when compared with the other treatments.

Egg Production and Quality Traits

Hens of T1 group and those exposed to FLASH20 had significantly ($P = 0.0233$) the highest egg laying rate and egg mass ($P = 0.0452$) in comparison with the other counterparts (Table 4). It is evident from the results presented in Table 4 that eggs produced from Rhode Island hens exposed to FLASH20 had significantly ($P = 0.0348$) the highest value of shell thickness (32.85 × 0.01 mm) followed by that of hens of the control (32.54 × 0.01 mm) and then that of those exposed to FLASH40 (30.62 × 0.01 mm). There were non-significant differences among the treatments in all of other egg quality (egg shape index, egg yolk index, Haugh units, shell strength, and egg components) and egg problem (floor eggs, cracked, and dirty eggs) traits (Table 4).

DISCUSSION

The reduction in MDA production in the group of birds exposed to FLASH20 could be attributed to the reduction in endogenous heat production by the birds as a result of the T_3 stimulation which decreases the heat production (Asal, 2013). FLASH20 has the ability to protect hen's body against oxidative stress and hence against increasing T-AOC and decreasing MDA level. MDA is present in lipoproteins; however, in the present study, MDA was found to decrease cholesterol levels as hens were kept under FLASH20. The importance of these antioxidant markers is due to their contribution in the clearance of superoxide and H_2O_2 to maintain the structure and function of the biological membranes (McCord, 2000). MDA is a major oxidation product of peroxidized polyunsaturated fatty acids, and increased MDA content is an important product of lipid peroxidation (Hassan et al., 2014). Rozenboim et al. (1999) documented that continuous lighting decreases the opportunity for rest and sleep, thereby increasing fear reaction and physiological stress. Hens supplied with intermittent light regimes have lower physiological stress, improved immune response, and increased sleep and rest (Classen et al., 2004; Farghly and Makled, 2015). The circadian rhythm is the physiological control of the metabolic activities of an individual by the light (Jácome et al., 2014). Light and dark cycles could practice hen's body to excrete hormones during a specific period (Navara and Nelson, 2007). The latter authors added that the circadian rhythm results in an adaptive temporal response, which allows individuals to adapt to the daily light–dark cycles in their house, to optimally time metabolism, physiology, and behavior each day. The increase in T_3 concentration leads to an elevation in luteinizing hormone, which is responsible for oviposition and ovulation (Siopes, 2007; Gumułka and Rozenboim, 2015) and then increasing HDP. It has been stated that T_3 is the major thyroid hormone regulating oxygen consumption and a metabolically more active substance than T_4 (Olanrewaju et al., 2013). Melatonin hormone is excreted during the dark times, and affects the production of different lymphocytes that are complementary to normal immune function by acting through thyroid hormones and increase the production

Table 2. Effect of intermittent light regime on blood parameters and antioxidant markers.

Traits	Treatments			SEM[1]	*P* value
	C	T1	T2		
Total proteins (g/dL)	5.04	5.48	5.12	0.49	0.6253
Albumin (g/dL)	2.84	3.09	2.93	0.28	0.3191
Globulin (g/dL)	2.20	2.39	2.19	0.33	0.7215
Albumin: globulin ratio	1.29	1.29	1.34	0.21	0.5122
Cholesterol (mg/dL)	133.23	129.18	132.24	5.88	0.4436
AST U/I	28.21	26.34	27.55	2.11	0.3681
ALT U/I	12.88	11.31	12.12	0.88	0.5133
T3 (ng/mL)	2.88	3.19	3.02	0.24	0.6194
T-AOC (nmol/mL)	2.17[b]	3.23[a]	2.68[a,b]	0.41	0.0221
MDA (nmol/mL)	9.23[a,b]	7.82[b]	10.12[a]	1.68	0.0435

C = Birds were exposed to constant light (16 h), T1 and T2 = birds were exposed to intermittent light for 20 and 40 min/h, respectively.
[1]SEM: standard error mean.
[a,b]Means with different superscripts in the same raw are significantly different ($P < 0.05$).

Table 3. Effect of intermittent light regime on egg production traits and feed conversion ratio.

Traits	Treatments			SEM[1]	*P* value
	C	T1	T2		
Body weight					
Initial BW (g)	1561.33	1542.53	1572.14	21.11	0.6353
Final BW (g)	1675.11	1718.51	1683.33	30.94	0.3856
Feed consumption and conversion					
FC (g/bird/d)	103.30	102.51	101.85	2.13	0.3726
FCR (g feed/g gain)	3.16[a]	2.98[b]	3.20[a]	0.12	0.0438

C = Birds were exposed to constant light (16 h), T1 and T2 = birds were exposed to intermittent light for 20 and 40 min/h, respectively.
[1]SEM: standard error mean.
[a,b]Means with different superscripts in the same raw are significantly different ($P < 0.05$).

Table 4. Effect of intermittent light regime on egg quality traits and egg problems.

Traits	Treatments			SEM[1]	*P* value
	C	T1	T2		
Egg production					
Egg laying rate (HDP, %)	68.71[a,b]	70.31[a]	66.65[b]	3.21	0.0233
Egg weight (g)	48.67	49.13	47.96	2.11	0.5644
Egg mass (g)	33.44[a,b]	34.54[a]	31.97[b]	1.98	0.0452
Egg quality					
Egg shape index (%)	77.56	77.31	76.88	3.29	0.7255
Egg yolk index (%)	52.03	52.66	51.92	2.75	0.4452
Haugh units	83.22	83.46	82.94	4.24	0.7833
Shell thickness (×0.01 mm)	32.54[a]	32.85[a]	30.62[b]	1.34	0.0348
Shell strength (kg/cm^2)	4.48	4.52	4.31	0.75	0.2735
Egg components (%)					
Albumen	57.00	56.79	57.04	2.16	0.3456
Yolk	31.92	32.16	32.11	1.32	0.3811
Shell	10.88	11.02	10.75	1.18	0.7365
Egg problems (%)					
Floor eggs	4.00	3.82	4.11	0.88	0.3254
Cracks and dirty	6.14	5.62	6.31	1.22	0.4639

C = birds were exposed to constant light (16 h), T1 and T2 = birds were exposed to intermittent light for 20 and 40 min/h, respectively.
[1]SEM: standard error mean.
[a,b]Means with different superscripts in the same raw are significantly different ($P < 0.05$).

of antibodies (Kliger et al., 2000; Abbas et al., 2007; Farghly, 2014).

In consistent with the present findings, El-Fiky et al. (2008) postulated that total protein and cholesterol concentrations were not different among the different lighting regimes. The current findings are in good agreement with those showed by Farghly (2014) and Farghly and Makled (2015), who reported non-significant differences in blood parameters of hens exposed to light flashes and those of the control. Shi

et al. (2007) concluded that there was no photoperiod that caused changes in T_3 concentration in Magang goose ganders. Onbaşılar et al. (2007) found that cholesterol levels did not differ significantly among different lighting groups. Olanrewaju et al. (2013) indicated that continuous exposure of broiler chickens to varying lighting regimes had a minor impact on blood parameters, whereas short photoperiod markedly affected most blood parameters without inducing physiological stress in broilers. On the other hand, Farghly et al. (2016) stated that there were significant differences in cholesterol concentration due to lighting regimes.

Some intermittent lighting regimes have been shown to enhance FCR (Ma et al., 2013; Farghly et al., 2016). As reported by Morris (2004), intermittent lighting regime offers a saving in FC related to the period of darkness in the day, and hence improving FCR. Laying hen appears to be stimulated to FC by a dusk period (Savory, 1980). The task of this feeding peak in response to simulated dusk would be that birds take the opportunity to fill their crop and prevent food deficit occurring during the dark (Savory, 1980; Bryant, 1987). Hence, hens could adjust their feeding activity according to the expected length of dark period.

Lighting regimes affected performance of egg production traits (Farghly, 2014; Farghly and Makled, 2015; Farghly et al., 2016). Changing the lighting regime is one of the most important management tools available for breeding laying hens. However, the present results suggest that laying hens may benefit from the systematic changes between light and dark. In the present work, the final BW of hens exposed to continuous lighting was the lightest among the treated groups. To keep high egg production level similar to that obtained by 16L: 8D constant regime, intermittent light must be administered between 4 and 10 h (Cavalchini et al., 1990). The increase in egg mass in hens exposed to Flash20 in the present work is mainly related to the increase in egg laying rate of the same group. In an earlier investigation carried out by Morris and Butler (1995), 2 intermittent lighting regimes for laying hens were applied: the biomittent system, using an asymmetric pattern of 0.25L: 0.75D for 16 h followed by 8D, and the traditional lighting regime (4[3L: 3D]). The biomittent system gave 2% fewer eggs with a 2% increase in egg weight than the conventional one. With applying 8 pulses of light per minute hourly after 8 h of continuous light, and when intensity of light was decreased to 5 or 1.25 lux, there was lack of response with intermittent lighting and no stimulus was transmitted to the photoperiodic mechanism (Mian, 2002). In the present investigation, we kept the light intensity throughout the experiment, so egg laying rate in FLASH20 was the highest. As indicated by Siopes (1999), intermittent lighting regime can be useful in increasing egg weight, but in the current results, egg weight remains the same. The present findings of egg production agreed with those of Lewis et al. (2004, 2007), Lewis and Gous (2006a,b), and Zhu et al. (2017), who stated that light regimes significantly influenced egg production. Also, Shen et al. (2012) indicated that egg production of intermittence lighting program of 8L: 4D: 4L: 8D was increased by 5.60% compared with the general lighting regime (16L: 8D). However, HDP was significantly higher in hens given longer photoperiod than those provided with the shorter one (Lewis et al., 2010). Similar results were observed by Lewis et al. (2010), who found non-significant influences on egg weight due to lighting periods. However, egg weight was significantly influenced by lighting regimes (Backhouse et al., 2005; Lewis and Gous, 2006a, 2006b). Ma et al. (2013) showed similar results to the present findings regarding FC. Significant influences were recorded on egg productivity due to different intermittent lighting regimes applied by Geng et al. (2014). In support of our results, Bahloul et al. (2014) indicated that the application of the short-constant lighting regime during the growth period and the intermittent one during the production stage was the best favorable to aid in an improvement in egg production and an increase in the egg mass. Similar results were also reported by Molino et al. (2015), who indicated that lighting programs influence egg production and egg mass. Farghly (2014) observed insignificant changes in egg weight, egg number, and hen day egg production among the experimental groupsby flash light. Farghly et al. (2016) showed that laying hens exposed to continuous lighting program had significantly higher egg production percentage than those kept under the intermittent one. In contrast to the present findings, Yuri et al. (2016) concluded that the use of intermittent lighting regimes (with 2 different photophases), for semi-heavy laying hens, reduces their productive performance and egg mass.

The current findings demonstrate that flash light did not exert different effects on egg quality traits except eggshell thickness in Rhode Island hens. Photoperiod is a factor that affects the egg quality of laying hens (Mohammed, 2016). The purpose of this work was to maintain and enhance egg production and egg quality, while saving electricity. Therefore, the lack of influences of flash lighting on egg quality (except egg shell thickness) is considered positive because birds maintained at the lowest period of electricity consumption achieved the same egg quality as those of the continuous lighting.

The biomittent system (0.25L: 0.75D for 16 h followed by 8D) gave a 3% increase in eggshell thickness than the conventional one at the end of the laying year (Morris and Butler, 1995). Due to the reduction in the physical activity during darkness, increasing resting and energy expenditure of activity is considerable (Ma et al., 2013; Farghly, 2014; Farghly and Makled, 2015; Yuri et al., 2016), and floor, cracks and dirty eggs were low in FLASH20. Light regime and duration are important factors that affect egg production and quality of laying hens (Lewis and Gous, 2006a,b). The present findings are consistent with the results obtained

by Molino et al. (2015), who showed changes in the eggshell thickness of Japanese quail eggs when comparing different lighting regimes.

The obtained findings of egg quality are in line with observations of Backhouse et al. (2005), who found that eggshell thickness was reduced for every hour increase in photoperiod. Also, Shen et al. (2012) claimed that eggshell thickness did not significantly differ among the lighting regimes. Leeson et al. (1982) reported that egg size, eggshell quality, and albumen quality, as assessed by Haugh units, were not affected by intermittent light regimes. Farghly (2014) found non-significant changes in egg shape index and Haugh units as affected by light flashes regime, while eggshell thickness was significantly affected. Farghly et al. (2016) observed that hens kept under the continuous light regime had significantly higher egg quality traits than those reared under intermittent light. Yuri et al. (2016) showed similar results, where egg quality traits were not changed due to intermittent lighting regimes. On the contrary, Mohammed (2016) reported significant changes in egg quality criterion due to different photoperiods. However, Lewis et al. (2004, 2007, 2010) and Lewis and Gous (2006a,b,c) reported significant changes in the proportion of floor eggs and the number of cracked and dirty eggs due to lighting regime effects. These authors also reported that the shorter photoperiods were associated with higher incidences of floor eggs and the production of more cracked and dirty eggs. Ma et al. (2013) showed comparable results to the present findings regarding cracked eggs.

It can be concluded from the findings of the present work that the intermittent light regime of 20 min/h (T1 group) was the most efficient in comparison with the other ones. This could be attributed to the superiority of hens exposed to intermittent light regime 20 min/h in egg production performance, FCR, shell thickness, and antioxidant markers. From the practical point of view, intermittent light regime of 20 min/h during laying period is highly recommended.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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