



Effect of various LED light color on the behavior and stress response of laying hens

S SULTANA¹, M R HASSAN², H S CHOE³, M I KANG⁴, B S KIM⁵ and K S RYU⁶

Chonbuk National University, Jeonju 561 756 Korea

Received: 17 October 2012; Accepted: 6 March 2013

ABSTRACT

The effects of various light emitting diode colors were observed on laying hen behavior. A total of 200 Hy-line brown laying hens were employed, and lighting was performed as follows; white (W, control; 16 h/d), red (R; 618–635 nm; 16 h/d), green (G; 515–535 nm; 16 h/d), blue (B; 455–470 nm; 16 h/d), red→green (R→G; 14 h→2 h/d), red→blue (R→B; 14 h→2 h/d), red→green→blue (R→G→B; 12 h→2 h→2 h/d) and red→blue→green (R→B→G; 12 h→2 h→2 h/d) light. Behaviors were recorded as frequencies except perching behavior, which was expressed as time per hour. Hens reared in R light were more active, as expressed by greater feather pecking, ground pecking, ground scratching, and tail wagging activity than those of hens in the B light treatment. Birds spent more time perching under the B light. Feather pecking and ground scratching behaviors decreased, and preening behavior increased in frequency with age of the laying hens. On the other hand, numerically higher H: L ratio and tonic immobility duration were obtained under the R→B treatment. These results may be useful to control the behavior of laying hens.

Key words: Age, Behavior, Laying hens, LED, Light color

Lighting is an important environmental factor that influences the physiology and behavior of chickens (Olanrewaju *et al.* 2006). The chicken eye is capable of discriminating light color due to 7 photoreceptors (1 rod and 6 cones) in the eye (Hart 2001).

In commercial market, LED lights are much more energy efficient and provide adequate illumination. Thus, conventional light based systems are being banned from 2012 and gradually replacing under the LED light in the Republic of Korea. Therefore, substantial effort needs to expend to find out which light colour minimizes the chickens normal behaviour. Birds reared in R light are more active, as expressed by the frequencies of pecking at the floor and wing stretching compared with those of birds under B and G light (Prayitno *et al.* 1997). Previous researchers have focused on hen laying performance (Kim *et al.* 2012) but little is known about the effects of monochromatic and combined light color on the behavior and stress of laying hen. Thus, the present

study was undertaken to evaluate different monochromatic lights and combinations of LED light colors and age on the behaviour and stress responses of laying hen.

MATERIALS AND METHODS

Birds feeding and management: A total of 200 Hy-line brown laying hens (29 weeks of age) were randomly housed in 8 light controlled rooms (2.6 × 1.9 m), and each room contained 25 birds at a density of 5 bird/m². Air temperature (21–24°C) and humidity (60–65%) were maintained with supplementary heating in winter. A ventilation fan in the roof of the central arena in each room extracted polluted air and circulated fresh air inside the pen. The floor was bedded with rice hulls (approximately 10 cm deep) and equipped with a long feeder, nipple drinker, perches, and laying nests. Hens had *ad lib.* access to feed and water, and diets were formulated (ME 2750 Kcal/kg and CP 16%) to meet the nutrient recommendations for poultry (NRC 1994). All management of the laying hens and experimental procedures were conducted in accordance with the Institutional Animal Care and Use Committee at Chonbuk National University, Korea.

Light treatments: The LED light treatments in a completely randomized design were: 1) red (R), 618–635 nm, 2) green (G), 515–535 nm, 3) blue (B), 455–470 nm, 4) control white (W), (fluorescent light), 5) red→green (R→G), 6) red→blue (R→B), 7) red→green→blue (R→G→B), and 8) red→blue→green (R→B→G) combination treatments.

Present address: ^{1,2}Ph. D student (snigdha_co@yahoo.com, mdrakibulhassan@gmail.com), ³Assistant professor, Department of Animal Biotechnology (hschoe@chonbuk.ac.kr); ⁴Professor, College of Veterinary Medicine, Chonnam National University, Korea; (mikang@chonnam.ac.kr); ⁵Professor, Department of Companion and Laboratory Animal Science, Kongju National University, Korea (bskim5247@hanmail.net); ⁶Professor, Department of Animal Science, Chonbuk National University, Korea (seon@jbnu.ac.kr).

Hens were subjected to a photoperiod of 16 h/day and light intensity was equalized (20 ± 0.21 Lx) fortnightly at the bird's head level using an illuminance meter T-10. The monochromatic R, G, B and W lighting treatments were performed from 6 AM to 10 PM daily. Therefore, the R→G treatment R light was provided for 14 h (6.00 AM to 8.00 PM) and then moved to 2 h of the G light (8.00 PM to 10.00 PM) daily. The R light in the R→B treatment was provided for 14 h (6.00 AM to 8.00 PM) and then moved to the B light for 2 h (8.00 PM to 10.00 PM) daily. The R light for the R→G→B treatment was provided for 12 h (6.00 AM to 6.00 PM) and then the G light was provided for 2 h (6.00 PM to 8.00 PM) and B light was provided again for 2 h (8.00 PM to 10.00 PM) daily. Thus, the R→B→G light treatment was provided daily consecutively similar to R→G→B. The light colors were changed by an automatic lighting software program. The LED light plate (38×24 cm) were hung 2 m above the floor litter and were pointed in a downward direction to lighten the pens. The electric voltages of the R, G, and B bulb devices were 2.0, 3.2, and 3.2 volts, respectively. The LED bulb devices had the same current, $I_F = 60$ mA.

Behavioral observations: Video data were observed at 2 stages from 29 to 36 weeks and from 41 to 48 weeks of age. Video was recorded for 3 consecutive days per week. The location and behavior of the birds were recorded at the same time for 4 h/day, 1 h in the morning (9.30–10.30 AM) and 3 h in the afternoon (5.30 PM–8.30 PM). The camera captured a full view of the pen. Behaviors were recorded for the following activities: feather pecking, preening, perching, dust-bathing, ground pecking, ground scratching, tail-wagging, and wing-flapping. The feather pecking, preening, dust bathing, ground pecking, ground scratching, tail wagging, and wing flapping behaviors were expressed as a frequency, and perching behavior was expressed as time per hour. The recorded video was analyzed at 5 min intervals (Prayitno *et al.* 1997) and the frequencies of these behaviors were recorded.

A bird was considered to be feather pecking when it was investigating another bird's feathers using their beak.

Preening was considered when manipulation of plumage with the beak was observed. A bird was “dust-bathing” when they scratched the floor and sprinkled the dust over the own body, along with fast shaking legs. We noted “perching” when a bird sat on a place above the soil level. Ground pecking was observed when a bird pecked the ground but not in or around the feeder. Extending both wings out from the body simultaneously and flapping of the wings were considered wing flapping. Ground scratching was considered when birds scratched the litter with their claws. Tail wagging was characterized by depressed tail feathers that moved rapidly from side to side.

At the end of the experiment, 10 blood samples per treatment were collected from the wing vein, smears were prepared, and lymphocytes were counted at $\times 40$ (oil immersion lens). H/L was calculated according to Wall *et al.* (2008). On the other hand, 10 birds per treatment were used and repeated three times and thus tonic immobility (TI) was measured as described by Sonatra *et al.* (2002).

Statistical analysis: The statistical analysis of all data was performed by analysis of variance using the General Linear Models procedures in SAS (2005). Significant differences among treatment means were identified by Duncan's new multiple-range test (Steel and Torrie 1980). A $P < 0.05$ was considered significant.

RESULTS AND DISCUSSION

Feather pecking: A behavioral analysis at 29–36 weeks failed to demonstrate a significant effect, but a higher frequency of feather pecking tended to be found in the R treatment than those in the B and R→B treatments due to R light, whereas other monochromatic light colors and their combinations showed an intermediate effect (Table 1). Therefore, this behavior was not altered by light color during the second phase of the study (41–48 weeks) (Table 3). Mohammed *et al.* (2010) reported that hens reared in the B light numerically increased feather pecking behavior. Therefore, these results are not consistent with our results may be due to the variation of light source and intensity. However, Lewis and Morris (2000) mentioned that light color

Table 1. Effect of LED light color on different laying hen behaviors at 29–36 weeks¹

Tr.	FP (n/b/h)			PR (n/b/h)			PC (min/h)			DB (n/b/h)		
	M	A	Mean	M	A	Mean	M	A	Mean	M	A	Mean
R	16.6	23.0	19.8	621.0	649.1	635.0	16.9 ^b	17.6 ^c	17.5 ^c	9.9	14.5	12.2
G	16.1	20.9	18.5	626.3	611.3	618.8	19.4 ^{ab}	20.9 ^{ab}	20.1 ^b	8.0	12.0	10.0
B	15.4	18.6	16.9	676.1	637.4	656.8	20.8 ^a	22.9 ^a	21.9 ^a	6.8	10.4	8.6
W	16.4	19.4	17.9	567.4	534.2	550.8	19.0 ^{ab}	20.9 ^{ab}	19.9 ^b	9.7	13.4	11.6
RG	16.3	22.4	19.4	617.3	640.9	629.1	17.8 ^b	19.1 ^b	18.8 ^{bc}	9.4	13.3	11.3
RB	15.8	18.4	17.1	609.0	433.2	521.1	17.8 ^b	20.2 ^b	18.9 ^{bc}	7.3	10.7	9.0
RGB	15.9	22.6	19.3	678.7	455.9	567.3	17.8 ^b	20.7 ^{ab}	19.3 ^b	8.3	12.2	10.3
RBG	15.7	19.9	17.8	644.6	549.9	597.3	17.3 ^b	19.9 ^b	18.5 ^{bc}	7.9	11.5	9.7
SEM	0.2	0.6	0.7	26.2	33.8	12.5	0.3	0.3	0.3	0.8	0.5	0.4

appears to have minimal influence on feather pecking due to R light would reach the hypothalamus more rapidly than that of B light.

Perching: The birds (29–36 weeks) spent significantly ($P<0.05$) more time perching under the B light treatment (Table 1) than that of the other monochromatic and combination treatments might be due to calming effect of the short wavelength (Prayitno *et al.* 1997). Similarly, birds tended to spend a longer time perching under the B light treatment during weeks 41–48, but the differences was not significant (Table 3). A direct comparison was not possible with the present results due to the lack of previous studies regarding the effects of LED light color on laying hen behavior. Thus, we speculate that the birds preferred to perch under short wavelength light compared to that of the long wavelength lighting conditions.

Ground pecking and scratching: Ground pecking and scratching behaviors responded significantly ($P<0.01$) to light color (both at 29–36 and 41–48 weeks) in which higher frequencies were obtained for R light than those for the B treatment.

The R→B, R→G→B, and R→B→G treatments showed intermediate effects (Table 2). Consequently, similar trends were also found at 41–48 weeks (Table 4). In the present experiment, we observed that these 2 behaviors were linked with each other. Birds frequently pecked the floor at the same time they were scratching than at other times in a day. In previous observations, Prayitno *et al.* (1997) stated that R light increases floor pecking behavior compared to that of B and G light and which was consistent with the present results.

Dust bathing and preening: Preening and dust bathing behavior were unaffected by light color during the 29–36 week video observations (Table 1) but tended to increase with decreasing wavelength of light. These trends also continued in the second phase (41–48 weeks) in which dust bathing and preening behavior of laying hens were not influenced by light color (Table 3). In present results, the majority of bird's time was involved in preening behavior due to their less aggressive nature. Birds frequently sat on a perch and manipulated their feathers. Thus, preening behavior frequencies were higher under the short wavelength (B) than that under the long wavelength (R). Dust bathing behavior

Table 2. Effect of LED light color on different laying hen behaviors at 29–36 weeks²

Tr.	GP (n/b/h)			GS (n/b/h)			TW (n/b/h)			WF (n/b/h)		
	M	A	Mean	M	A	Mean	M	A	Mean	M	A	Mean
R	349.4	406.1 ^a	377.76 ^a	43.80	72.6 ^a	58.2 ^a	21.0	25.3 ^a	23.2 ^a	18.0	15.7	16.9
G	315.0	348.1 ^{ab}	331.56 ^{ab}	44.40	65.6 ^{ab}	55.0 ^{ab}	13.9	21.7 ^{ab}	17.8 ^b	16.1	15.4	15.7
B	231.0	252.7 ^c	241.86 ^c	31.56	51.7 ^b	41.6 ^c	16.7	19.9 ^{bc}	18.3 ^b	15.4	15.7	15.5
W	342.0	334.6 ^{abc}	338.28 ^{ab}	36.00	63.6 ^{ab}	49.8 ^{abc}	17.7	17.0 ^{bc}	17.4 ^b	15.7	14.4	15.1
RG	327.0	385.1 ^{ab}	356.04 ^a	44.04	63.4 ^{ab}	53.7 ^{abc}	20.2	19.8 ^{bc}	19.9 ^{ab}	16.4	13.4	14.9
RB	255.8	340.1 ^{abc}	297.96 ^{abc}	36.72	49.8 ^b	43.3 ^c	13.4	17.6 ^{bc}	15.5 ^b	16.8	17.3	17.0
RGB	313.4	333.7 ^{abc}	323.59 ^{abc}	42.00	61.7 ^{ab}	51.8 ^{abc}	21.7	19.3 ^{bc}	20.5 ^{ab}	18.0	12.7	15.4
RBG	245.0	303.8 ^{bc}	274.44 ^{bc}	39.00	49.6 ^b	44.3 ^{bc}	18.4	15.7 ^c	17.0 ^b	15.0	14.3	14.6
SEM	14.4	11.9	10.01	1.32	2.04	2.06	0.7	0.60	0.79	3.60	0.48	0.23

^{1,2}SEM, standard error of mean; Tr, treatment; FP, feather pecking; PR, preening; PC, perching; DB, dustbathing; GP, ground pecking; GS, ground scratching; TW, tail wagging; WF, wing flapping; M, morning; A, afternoon; m/h, minute/hour; n/b/h, number/bird/hour; R, red; G, green; B, blue; W, white; RG, red→green; RB, red→blue; RGB, red→green→blue; RBG, red→blue→green; ^{abc}values in a column with no common superscripts differ significantly ($P<0.05$).

Table 3. Effect of LED light color on different laying hen behaviors at 41–48 weeks¹

Tr.	FP (n/b/h)			PR (n/b/h)			PC (min/h)			DB (n/b/h)		
	M	A	Mean	M	A	Mean	M	A	Mean	M	A	Mean
R	15.4	20.8	18.1	651.7	715.1	683.4	18.1	19.4	18.8	9.1	11.6	10.4
G	11.2	20.0	15.6	600.7	636.7	618.7	19.4	20.9	20.1	7.6	10.3	8.9
B	7.4	11.0	9.2	720.7	681.0	700.9	21.0	21.1	21.0	5.6	7.9	6.8
W	12.2	18.5	15.4	595.4	574.8	585.1	19.0	20.9	19.9	9.9	13.1	11.5
RG	10.1	17.9	13.9	659.6	669.4	664.5	17.8	19.8	18.8	9.7	10.2	9.9
RB	9.2	16.6	12.9	714.7	632.3	673.5	17.8	20.4	19.1	6.9	8.9	7.9
RGB	11.8	19.1	15.4	721.4	507.1	614.3	17.8	20.6	19.3	9.2	12.2	10.7
RBG	9.1	16.9	13.0	659.2	668.3	663.7	16.9	20.7	18.6	7.4	9.0	8.2
SEM	0.72	0.84	1.44	27.84	29.16	17.70	0.61	0.56	1.67	0.60	0.72	0.69

Table 4. Effect of LED light color on different laying hen behaviors at 41–48 weeks²

Tr.	GP (n/b/h)			GS (n/b/h)			TW (n/b/h)			WF (n/b/h)		
	M	A	Mean	M	A	Mean	M	A	Mean	M	A	Mean
R	305.3	377.4 ^a	341.3 ^a	42.8	59.2 ^a	51.0 ^a	20.8	22.6 ^a	21.7 ^a	16.8	15.8	16.3
G	295.6	327.7 ^{ab}	311.6 ^{ab}	35.4	57.1 ^{ab}	46.3 ^{ab}	15.7	21.1 ^{ab}	18.4 ^b	15.9	15.2	15.6
B	232.6	255.1 ^c	243.8 ^c	22.8	35.6 ^{ab}	29.2 ^c	14.5	19.4 ^{bc}	16.9 ^b	14.4	14.9	14.6
W	301.2	355.4 ^{abc}	328.3 ^{ab}	41.4	51.9 ^{ab}	46.7 ^{abc}	16.8	17.4 ^{bc}	17.1 ^b	15.1	14.2	14.6
RG	297.4	339.5 ^{ab}	318.4 ^a	35.4	51.4 ^b	43.4 ^{bc}	21.8	18.7 ^c	20.3 ^{ab}	15.9	13.9	14.9
RB	253.3	280.2 ^{abc}	266.7 ^{abc}	26.6	42.9 ^b	34.8 ^c	20.4	16.3 ^{bc}	18.4 ^b	15.9	16.4	16.2
RGB	280.2	288.5 ^{abc}	284.3 ^{abc}	31.3	40.2 ^{ab}	35.8 ^{abc}	20.3	18.6 ^{bc}	19.4 ^{ab}	15.5	12.0	13.7
RBG	270.2	267.5 ^{bc}	268.9 ^{bc}	27.1	36.1 ^b	31.6 ^{bc}	19.4	15.5 ^c	17.5 ^b	15.4	13.7	14.5
SEM	7.80	12.96	9.96	1.92	2.76	3.60	0.6	0.84	0.49	0.36	0.36	0.42

^{1,2}SEM, standard error of mean; Tr, treatment; FP, feather pecking; PR, preening; PC, perching; DB, dustbathing; GP, ground pecking; GS, ground scratching; TW, tail wagging; WF, wing flapping; M, morning; A, afternoon; m/h, minute/hour; n/b/h, number/bird/hour; R, red; G, green; B, blue; W, white; RG, red→green; RB, red→blue; RGB, red→green→blue; RBG, red→blue→green; ^{abc}values in a column with no common superscripts differ significantly ($P<0.05$).

Table 5. Effect of time of the day and age on the behaviour of laying hen

Treatment	FP(n/b/h)	PR(n/b/h)	PC(m/h)	DB(n/b/h)	GP (n/b/h)	GS(n/b/h)	TW(n/b/h)	WF(n/b/h)
Time of the day								
Morning	13.4 ^b	647.7	17.3 ^b	8.3 ^b	288.4 ^b	36.3 ^b	18.3	16.0 ^a
Afternoon	19.1 ^a	599.8	19.7 ^a	11.3 ^a	324.7 ^a	53.3 ^a	19.1	14.7 ^b
SEM	0.74	12.41	0.54	0.39	8.02	2.19	0.48	0.24
Bird age								
29–36 wks	18.4 ^a	597.0 ^b	19.4	10.4	317.7	49.7 ^a	18.7	15.7
41–48 wks	14.2 ^b	650.5 ^a	19.5	9.3	295.4	39.8 ^b	18.7	15.1
SEM	0.71	12.56	0.39	0.38	10.01	2.06	0.49	0.23

SEM, standard error of mean; FP, feather pecking; PR, preening; PC, perching; DB, dustbathing; GP, ground pecking; GS, ground scratching; TW, tail wagging; WF, wing flapping; m/h, minute/hour; n/b/h, number/bird/hour; ^{abc}values in a column with no common superscripts differ significantly ($P<0.05$).

was not influenced by the light color due to its maintenance function, which improves feather condition by dispersing lipids (Van Liere and Wiepkema 1992).

Wing flapping and tail wagging: Although wing flapping behavior (29–36 weeks) was not influenced by light color, mean tail wagging behavior increased ($P<0.05$) in the R color treatment but was lower in combination with B (R→B color, Table 2). R light stimulated tail wagging behavior compared to that of wing flapping behavior at 41–48 weeks (Table 4). It might be due to the better visual sensitivity in chickens at the R compared with the B end of spectrum (Prayitno *et al.* 1997). The present results showed that those wing flapping and tail wagging behaviour were also influenced by the nearest neighbours activity.

Effects of time-of-day and age on the behaviour of laying hen: In the present experiment time of the day have also effect on the behaviour of laying hen (Table 5). Within the time budget, feather pecking, dustbathing, ground pecking and ground scratching frequency were significantly ($P<0.05$) higher in the afternoon than that of morning might be due to birds were spent their time for feeding and seeking their nest

and laid of eggs in the morning (Barbosa 2005). On the other hand, longer perching time was also found in the afternoon. In contrast, higher number of frequency of wing flapping was obtained in the morning. In the floor rearing system, there is no work on different LED light colour on the laying hen's behavior which complicates direct comparison of day time on laying hens behavior.

Bird age also influenced laying hen behavior (Table 5). Therefore, feather pecking and ground scratching behaviors decreased and preening behavior increased with laying hen age ($P<0.05$). Feather pecking tendency was decreased with the age of hens may have been due to decreased bird activity and which corresponds with the findings of Savory and Mann (1997). In another experiment, Anderson *et al.* (2005) mentioned that feather pecking was increased at 26–34 and 64 weeks of age. In the present observations, perching time and preening behaviors were positively linked with each other. Therefore, the increased preening frequency with bird age may have been due to a decrease in activity and increased perching time of laying hens.

But, ground pecking, dust-bathing, tail wagging, and wing

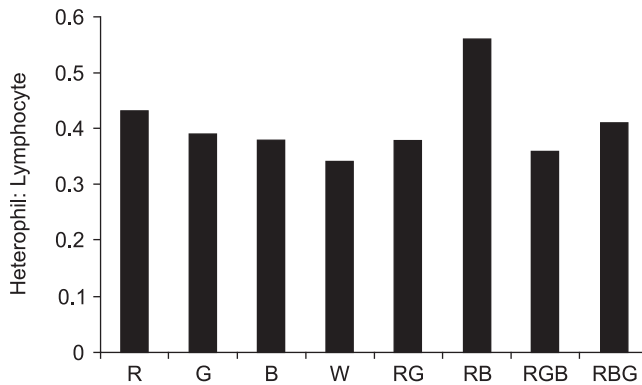


Fig. 1. Effect of LED light color on the H: L ratio of laying hen.

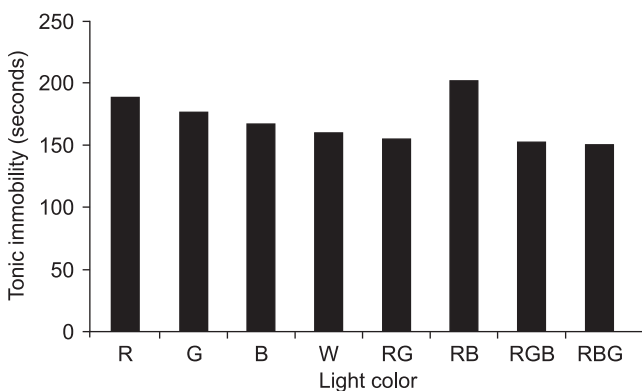


Fig 2. Effect of LED light color on tonic immobility of laying hen.

flapping behaviors were not influenced by age of the laying hens. Thus, the birds spent more time perching during the 41–48 week period than that during the 29–36 week period.

Heterophil: Lymphocyte ratio and Tonic immobility: Both H: L ratio and TI duration were not significantly affected by the light color (Figs 1 and 2) but numerically higher trends were obtained under the R→B and R treatments and lower in B treatment. Similar to the present findings, Scott and Siopes (1994) have reported that light color had no effect on H: L ratios. No scientific literature dealing directly with light color effects on tonic immobility duration in the scientific literature. However a direct comparison was not possible with the present results.

This study indicated that the ground pecking, ground scratching, and tail wagging behaviors were enhanced by R light, whereas B light decreased the movement and motivated perching behaviors, but combinations of light color did not play any additional role. Feather pecking and ground scratching behaviors decreased and preening behavior increased with age of the laying hen. On the other hand, different LED light color treatments did not influenced the H: L ratio and tonic immobility durations of laying hen.

REFERENCES

- Anderson K E, Davis G S, Jenkins P K and Carroll A S. 2004. Effects of bird age, density, and molt on behavioral profiles of two commercial layer strains in cages. *Poultry Science* **83**: 15–23.
- Barbosa F J A D. 2005. 'Welfare Evaluation by Image Analysis of Laying Hens in Different Houses Systems and Environmental Conditions.' MSc (Physics). Escola Superior de Agricultura "Luiz de Queiroz", Universidade de São Paulo, Piracicaba.
- Hart N S. 2001. Variations in cone photoreceptor abundance and the visual ecology of birds. *Journal of Comparative Physiology A* **187**: 685–97.
- Kim M J, Hossan M S, Nazma A, Jae C N, Han B T, Hwan K K, Dong W K, Hyun S C, Hee C C and Ok S S. 2012. Effect of monochromatic light on sexual maturity, production performance and egg quality of laying hens. *Avian Biology Research* **5**: 69–74.
- Lewis P D and Morris T R. 2000. Poultry and colored light. *World's Poultry Science Journal* **56**: 189–207.
- Mohammed H H, Grashorn M A and Bessei W. 2010. The effects of lighting conditions on the behavior of laying hens. *European Poultry Science* **74**: 197–202.
- NRC 1994. *Nutrient Requirements of Poultry*. 9th revised edn. National Academy of Science. Washington DC.
- Olanrewaju H A, Wongpichet S, Thaxton J P, Dozier W A and Branton S L. 2006. Stress and acid-base balance in chickens. *Poultry Science* **85**: 1266–74.
- Prayitno D S, Phillips C J C and Stokes D K. 1997. The effects of colour and intensity of light on behaviour and leg disorders in broiler chickens. *Poultry Science* **76**: 1674–81.
- Sanotra G S, Damkjer L J and Vestergaard K S. 2002. Influence of light-dark schedules and stocking density on behavior, risk of leg problems and occurrence of chronic fear in broilers. *British poultry Science* **43**: 344–54.
- SAS 2005. *SAS user's guide*. Statistical Analysis System Institute. Ver. 9.1 edn. Inc., Cary NC.
- Savory C J and Mann J S. 1997. Is there a role for corticosterone in expression of abnormal behaviour in restricted-fed fowls? *Physiology and Behavior* **62**: 7–13.
- Scott R P and Siopes T D. 1994. Light color: effect on blood cells, immune function and stress status in turkey hens. *Comparative Biochemistry and Physiology* **108A**: 161–68.
- Steel R G D and Torrie J H. 1980. *Analysis of Covariance. Principles and Procedures of Statistics: A Biometrical Approach*. 401–37. McGraw-Hill, New York.
- Van Lier D W and Wiepkema P R. 1992. Effects of long-term deprivation of sand on dustbathing behaviour in laying hens. *Animal Behaviour* **43**: 549–58.
- Wall H, Tauson R and Elwinger K. 2008. Effects of litter substrate and genotype on layers' use of litter, exterior appearance, and heterophil: lymphocyte ratios in furnished cages. *Poultry Science* **87**: 2458–65.
- Xie D, Li J, Wang Z X, Cao J, Li T T, Chen J L and Chen Y X. 2011. Effects of monochromatic light on mucosal mechanical and immunological barriers in the small intestine of broilers. *Poultry Science* **90**: 2697–2704.