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Effect of light colour on egg production and egg contamination

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ABSTRACT: The objective of this study was to compare the performance of laying hens, quality of air in poultry house, and microbial contamination of eggshell in laying hens kept under blue, green, red, and yellow light colour in enriched cages. The daily photoperiod consisted of 15 h light, light intensity of 10 lx at bird head level. The laying performance characteristics (hen-day egg production, mortality, and egg weight) were not affected by light colour. Similarly, microbial contamination of the air was not significantly different related to the light colour. There were significant interactions in eggshell contamination between the position of the cage floor and light colour in *Escherichia coli* ($P \leq 0.042$) and *Enterococcus* ($P \leq 0.019$). The highest number of *Escherichia coli* was detected in eggs from hens housed on the middle floor given yellow light (6.06 log colony forming units (cfu)/eggshell) and the lowest values (3.30 log cfu/eggshell) on the upper floor also under yellow light colour. Similar results were observed in *Enterococcus,* where the highest contamination was on the middle floor under yellow light colour (5.26 log cfu/eggshell), while the lowest contamination (2.45 log cfu/eggshell) was found on the upper floor under blue colour. The results of our study indicate that the light colour has a minor effect on microbial contamination but the significant influence was in the floor position. The highest microbial egg contamination was found on eggs from the middle floor.

Keywords: monochromatic lights; production performance; microbial contamination, laying hens

INTRODUCTION

Light is an important environmental factor that influences the behaviour, egg production, and health of laying hens, therefore, artificial illumination (light duration and light intensity) is widely used to increase the reproductive performance of laying hens in modern poultry houses (Er et al. 2007).

The physiological action of light happens when it is percepted by eye and converted into nerve impulses that are sent to the brain. The brain then coordinates the stimulus to influence the pituitary gland to secrete the necessary hormones for ovulation (Lewis and Morris 2000). The chicken eye is superior to the livestock eye and can discriminate light colour (Prescott and Wathes 1999). Furthermore, it can see a broader portion of the light spectrum compared with humans (380 to 760 nm) (Prescott and Wathes 1999). Both retinal and extra-retinal photoreceptors are responsive to red light, which stimulates gonadal development (Woodardet et al. 1969; Pyrzaket et al. 1987). Retinal cone cells contain coloured oil droplets that filter light and pass the signal to photoreactive pigments (Bowmaker and Knowles 1977). These cone cells respond maximally to violet, blue, green, and yellow spectrum (Dartnall et al. 1983).

Many physiological processes in poultry are influenced by light. Egg production and quality may

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be affected by visible spectrum emitted by the light source, and some colours may be more stimulating than others (Nicholls et al. 1988). There are limited data on the effect of light colour on laying hens performance and egg quality. For example, the greatest number of eggs was produced in a group treated with red light, and eggs laid under blue or green lights were consistently heavier than those laid under red light. The eggshell strength in green light was significantly higher than that in other lights (Pyrzak et al. 1987). In contrast, the reports of Woodard et al. (1969) for quails and Rozenboim et al. (1998) for chickens suggested that egg weight was not affected by light colour. Pyrzak and Siopes (1986) found no differences in maturity of pullets or egg production in force-moulted hens reared under blue or red monochromatic light compared with conventional incandescent light.

There are two possible ways in which bacteria may infect egg shells: vertically or horizontally. Vertical transmission occurs in the reproductive organs of infected hens mainly from infection of ovaries by systemic infection or ascending infection from contaminated cloaca into the vagina and lower regions of the oviduct (Keller et al. 1995; Miyamoto et al. 1997). Horizontal transmission occurs when eggs are subsequently exposed to a contaminated environment and microorganisms penetrate the eggshell. Eggs are potentially contaminated by any surface with which they come into contact. Sources of bacterial contamination of the shell include caging material, nesting materials, water, hands, broken eggs, blood, insects, and transport belting though dust, soil, and faeces (Board and Tranter 1995; Ricke et al. 2001; Davies and Breslin 2003). The bacterial contamination of eggshells can be affected by several factors such as e.g. the concentration of bacteria in the air of the poultry house (De Reu et al. 2005a).

Bacteria in the air of animal housing are assumed to have an impact on the health of humans and animals as well as on the environment. The bacterial count in poultry housing systems is particularly high in comparison to that of pigs and cattle. Dust concentrations in poultry houses vary from 0.02 to 81.33 mg/m^3 for inhalable dust and from 0.01 to 6.5 mg/m^3 for respirable dust (Ellen et al. 2000). Houses with caged laying hens showed the lowest dust concentrations, i.e. less than 2 mg/m^3, while the dust concentrations in other housing systems, e.g. perchery and aviary systems, were often four to five times higher. In alternative systemsm where the birds move freely in their environment, a significant amount of dust originating from litter is created, having as a consequence air contaminated with microorganisms and endotoxins (Hartung 1994; Wathes 1994). In some studies the total count of aerobic bacteria in the air of poultry houses was proven to be positively correlated with the initial bacterial eggshell contamination in the henhouse (Protais et al. 2003; De Reu et al. 2005b). The averages of 4 log colony forming units (cfu)/m^3 air for the conventional and furnished cages were found compared with a 100 times higher average (> 6 log cfu/m^3) for aviary housing systems (De Reu et al. 2010). Takai et al. (1998), Ellen et al. (2000), and Guillam et al. (2007) also reported higher dust concentrations in perchery and aviary systems than in cage poultry houses.

Prayitno et al. (1997) investigated the effect of light colour on the behaviour of meat chickens and confirmed that birds in red light spent more time in aggressive interaction, pecking at the floor, and wing stretching in comparison with green or blue light. They also confirmed that birds in the white light spent longer time by sleeping and walking activity, whereas birds in the green and blue lights spent relatively longer by sitting and dozing, respectively. It is likely that differences in activity of birds in different lights could also influence the amount of dust in the house with the most active birds stirring up the greatest amount of dust as indicated by the study of Ellen et al. (2000). However, information about the effect of light colour on microbial contamination in the air and on microbial contamination of eggs in the literature is missing.

The aim of the present study was to evaluate the effect of light colour on air quality in hen house, performance of laying hens, and the eggshell microbial contamination.

MATERIAL AND METHODS

An experiment with ISA Brown hens from 22 to 75 weeks of age was carried out. They were placed in four houses (14 400 laying hens per house) in identical environmental conditions with colour lights. Laying hens were housed in enriched cages (20 hens per cage, 750 cm^2 per hen) located on three floors. The following light colours were used: blue, green, red, and yellow. The light sources were light emitting diodes (LED) placed above feeders

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throughout the length of cages. The daily photoperiod consisted of 15 h light, with an intensity of 10 lx at bird head level. Laying hens were fed identical commercial feed mixtures, N1 in weeks 20–40 and N2 from 41 weeks of age. Feed and water were supplied *ad libitum*. Microclimate conditions were in accordance with laying hens' requirements.

Egg production and mortality were recorded daily. Egg weight was evaluated over a four-week interval, 1000 eggs from each group at each collection time using the method of Englmaierova et al. (2013).

Air microbial composition was determined using the Air Sampler MAS-100 Eco® (MBV AG, Stäfa, Switzerland). The total numbers of microorganisms (TNM), *Escherichia coli* (EC), and *Enterococcus* (E) per cubic metre of air were analyzed. Air samples were collected every four weeks during the whole laying cycle. The resulting airflow was transformed onto a standard Petri dish containing agar. For TNM determination Standard plate count agar, for EC determination Mac-Conkey agar, and for E determination Slanetz Bartley agar (all Oxoid™; Thermo Scientific, Tewksburg, USA) were used. Petri dishes were incubated for 120 h at 30°C (TNM) and for 48 h at 37°C (EC and E).

The eggs for analyses of microbial contamination of eggshell were collected once a month. Six eggs from each light colour (2 eggs from upper floor, 2 eggs from middle floor and, 2 eggs from lower floor) in one collection were analyzed, in total 120 eggs. Microbial analysis of the eggshell surface was performed on fresh eggs according to Englmaierova et al. (2014). The eggs were sampled by hand (wearing clean gloves), placed on a clean underlay. The eggs for analyses of shell contamination were placed in sterile plastic bags with 10 ml of sterile saline peptone (9 g sodium chloride, 1 g peptone, 1000 ml distilled water), in which the eggs were thoroughly rinsed. A dilution series for each egg was produced by adding 1 ml of solution (10^0, 10^{-1}, 10^{-2}, 10^{-3}, 10^{-4}, 10^{-5}). The microorganism analysis was carried out by standard agar methods. The number of *Escherichia coli* was monitored using Mac-Conkey agar, the number of *Enterococcus* using Slanetz Bartley agar, and the total number of microorganisms using Standard plate count agar (all Oxoid™; Thermo Scientific). Plates with Mac-Conkey agar and Slanetz Bartley agar were then incubated for 48 h in an incubator at 37°C. Standard plate count agar was incubated at 30°C for 120 h. Typical colony forming units (cfu) on eggshell were calculated on Petri plates after incubation.

Data for performance characteristics and microbial contamination in the air were statistically evaluated using one-way analysis of variance (ANOVA), the GLM procedure of SAS (Statistical Analysis System, Version 9.2, 2003). The significance of differences between groups was tested by the Duncan's Multiple Range Test. Data for egg contamination were processed by two-way ANOVA with interaction between floor number and light colour. The value of $P \leq 0.05$ was considered significant for all measurements.

RESULTS AND DISCUSSION

The hen-day egg production ranged from 87.01% in red light colour to 83.90% in green light colour (Table 1) and was not significantly different. These results correspond with those of Pyrzak and Siopes (1986) who did not observe any effect of light colour on egg production. However, Pyrzak et al. (1987) concluded that the significantly higher hen-day production was observed in layers kept in red light and the lowest hen-day production was observed in layers kept in blue light colour. The significantly highest hen-day production was observed in laying hens kept in red light (Min et al. 2012) and white light (Borille et al. 2013). Hassan et al. (2013) indicated that egg production was similar

Table 1. Performance of laying hens under different light colour

Characteristic	Light colour				RMSE	Significance
	blue	green	red	yellow		
Hen-day egg production (%)	84.77	83.90	87.01	86.75	9.37	ns
Mortality (%)	14.30	13.19	12.65	13.01	–	–
Egg weight (g)	58.86	58.97	58.89	58.89	2.59	ns

RMSE = Root Mean Squared Error, ns = not significant

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in white, green, and blue light colour. According to Lewis and Morris (2000) the penetration of the red wavelength in the hypothalamus is more sexually stimulating than green or blue wavelength. The reason for the positive effect of red light on egg production could be attributed to the more efficient penetration of light of longer wavelengths (towards the orange-red spectrum) through the skin and skull than of short wavelengths (towards blue-green spectrum) leading to improvement of the reproductive performance of birds (Solangi et al. 2004). Hassan et al. (2013) explained that the improving in average egg production when red light is used might be attributed to the elevation of serum follicle stimulating hormone and luteinizing hormone concentrations leading to the increase of ovarian follicle number.

The lowest mortality was recorded in laying hens housed in red light (12.65%). Conversely, the highest mortality was found in blue light (14.30%). Mohammed et al. (2010) found that under blue light activities of walking, feather pecking, and aggression were higher. Savory and Mann (1999) noted that pecking and cannibalism may be more likely in groups where activity levels are high. Perhaps hens searched for a way to compensate light inconvenience. It may also be related to higher mortality of hens in blue light colour. It has been reported that there is less cannibalism in laying hens under red light than under green or white light (Bowlby 1957; Schumaier et al. 1968), presumably because the birds cannot see the blood stimulant under red light, however, this behaviour is not normally observed in meat chickens. Huber-Eicher et al. (2013) also reported that red light reduced aggressiveness compared with white light (green was intermediate).

Egg weight values in all light colours ranged between 58.86 and 58.89 g and no significant differences were recorded. These results are in agreement with Woodard et al. (1969) and Rozenboim et al. (1998) in Japanese quail and laying hens, respectively. Rozenboim et al. (1998) confirmed that in laying hens the light colour did not affect the average egg weight during the laying period. Our results correspond also with the studies of Freitas et al. (2010) and Borille et al. (2013) who confirmed that egg weight was not influenced by light colour. However, Pyrzak and Siopes (1986) reported that turkey hens kept in red light produced heavier eggs than those on other light treatments. Likewise, Pyrzak et al. (1987) reported that eggs laid under blue light were consistently larger than those produced under red light. Er et al. (2007) observed that egg weight was lower in red light compared to blue and incandescent light. However, Borille et al. (2013) reported that egg weight usually depends particularly on hen's age and on nutritional factors rather than on light colour.

Results of the microbial contamination of the air are provided in Table 2. There was no effect of light colour on air contamination. In all measurements, TNM, EC, and E had low variability. There are no data in literature about the effect of light colour on microbial air contamination; however, we assume that the numerically higher volume of TNM under blue light could be the result of increased dustiness, which was probably due to higher activity of laying hens on this treatment. The available data are especially about the impact of housing system on air contamination. Aerial dust monitoring showed that the dust concentration was higher in on-floor hen houses than in conventional cage poultry houses (Huneau-Salaun et al. 2010). This poor microbiological air quality in alternative housing systems may affect the bacterial concentration on the eggs (Quarles et al.

Table 2. Effect of light colour on microbial contamination of air

Light colour	Bacterial strain (log cfu/m^3)		
	Escherichia coli	*Enterococcus*	total number of microorganisms
Blue	4.78	4.09	5.20
Green	4.98	3.97	5.08
Red	4.94	4.09	5.15
Yellow	4.97	3.99	5.06
RMSE	0.44	0.25	0.34
Significance	ns	ns	ns

RMSE = Root Mean Squared Error, ns = not significant, cfu = colony forming units

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Table 3. Effect of the position of the cage floor and light colour on the microbial contamination of eggshell

Floor	Light colour	Bacterial strain log (cfu/eggshell)		
		Escherichia coli	*Enterococcus*	total number of microorganisms
Upper	blue	5.08^{ab}	2.45^{c}	4.91
	green	4.88^{b}	4.02^{abc}	5.90
	red	4.85^{b}	4.07^{abc}	5.58
	yellow	3.30^{c}	3.01^{c}	5.39
Middle	blue	5.90^{ab}	5.02^{ab}	6.14
	green	5.44^{ab}	4.72^{ab}	5.94
	red	5.76^{ab}	4.96^{ab}	6.04
	yellow	6.06^{a}	5.26^{a}	6.61
Lower	blue	5.77^{ab}	5.01^{ab}	6.25
	green	5.47^{ab}	4.84^{b}	6.03
	red	4.74^{b}	3.38^{c}	6.00
	yellow	5.72^{ab}	5.07^{ab}	6.06
RMSE		1.18	1.27	0.76
Significance				
Floor		< 0.001	< 0.001	< 0.001
Light colour		ns	ns	ns
Floor × light source		0.042	0.019	ns

RMSE = Root Mean Squared Error, ns = not significant, cfu = colony forming units

$^{a–c}P \leq 0.05$

1970). The degradation of air quality in alternative systems may be due to the presence of litter and to the greater activity of hens (walking, flying, foraging). In addition, the natural ventilation systems in the alternative hen houses, in contrast to the dynamic ventilation systems in the caged buildings, could lead to a lower ventilation rate and thus a higher rate of dust concentration (Takai et al. 1998; Radon et al. 2001).

The microbial contamination was evaluated in each light colour and cage floor position (Table 3). There was a significant interaction between the cage floor and the light colour in EC and E. The highest number of EC was detected on the middle floor under yellow light colour whereas the lowest was also under yellow light, but on the upper floor (3.30 log cfu/eggshell). For E contamination, the significantly highest ($P \leq 0.019$) concentration was also under yellow light colour on the middle floor, however, the lowest was under blue light on the upper floor. There was a significant ($P < 0.001$) effect of the cage floor position on the number of TNM, EC, and E. The highest contaminations of eggshell were detected on the middle floor while the lowest were detected on the upper floor. Light colour has no significant effect on eggshell contamination. Only ultraviolet radiation at 254 nm is well known and documented for its use to kill various types of microorganisms, such as bacteria, yeasts, molds, fungi, and viruses (Chavez et al. 2002; Coufal et al. 2003). Significantly higher contamination of eggs on the middle cage floor could possibly be due to higher concentrations of dust at this level. As contamination of the eggshell appears to depend on the cleanliness of the surface on which the egg is laid (Harry 1963), the accumulation of dust and egg dirt in the cages or nests during the production period is likely to depress eggshell cleanliness and increase bacterial load. In addition, it was observed that the dust concentration in the air of the poultry houses increased during the laying period due to the accumulation of settled dusts in the hen houses (Huneau-Salaun et al. 2010).

CONCLUSION

Results of the present study showed that the light colour did not affect hen-day egg production, mortality or egg weight. Also the effect of light colour had no significant effect on the microbial

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contamination of the air in the hen house. On the other hand, significant interactions were observed in egg contamination between floor position and light colour in EC and E. The floor position significantly affected the number of microorganisms on eggshell. The highest contamination of egg shell was detected on the middle floor, probably due to the higher concentration of aerial dust that could be affected, amongst other things, by the activity of birds on the surrounding cage floors.

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