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Original Article *Genetics*

The Effect of colour temperature of light on egg production parameters and gene expression pattern of Heat Shock Protein 27 in layers

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ABSTRACT

Objective: To evaluate the effects of different light colour temperatures on egg production parameters and heat shock protein gene expression (HSP 27) in Fayoumi chicken layers. Animals: 165 Fayoumi healthy pullets (17 weeks).
Design: Randomized-controlled experimental study.
Procedures: The birds were exposed to natural day light with photoperiod 12L: 12D, from 19th week, lighting schedule was gradually increased half an hour every week till it reached 16L:8D lighting schedule at laying at 24 weeks of age. At laying, the birds were allocated into three groups at a density of 9 birds/m2. The first group (control group) was exposed to cool white LED light (day light) (6500 kelvin). The second group was exposed to very cool white LED light (sky blue light) (10000 kelvin). The third group was exposed to warm white LED light (yellow light) (2700 kelvin) till the end of the experiment. Eggs were collected daily; egg number, weight and mass were recorded for each group. Liver samples were collected for RNA extraction.
Results: The results showed that egg number, egg weight and egg mass were significantly increased in blue light group ($P \leq 0.05$) and HSP 27 gene expression was significantly upregulated ($P \leq 0.05$) in this group of chicken in comparison to the control group.
Conclusion and clinical relevance: It is concluded that the blue LED light may be suitable for use in layer farms to optimize reproductive efficiency of birds.

Keywords: Kelvin, Heat shock protein, Real Time PCR.

1. INTRODUCTION

Heat shock proteins (HSP) are a family of proteins that are produced by cells in response to exposure to stressful conditions. They were first described in relation to heat shock, but are now known to be also expressed during other stressful situations including exposure to cold, UV light, and during wound healing or tissue remodeling [1]. They are named according to their molecular weight. For example, *HSP 60*, *HSP70* and *HSP 90* refer to families of heat shock proteins on the order of 60, 70, and 90 kilodaltons in size, respectively. A conserved protein binding domain of approximately 80 amino-acid of alpha crystalline are known as small heat shock proteins *(sHSP)* [2]. HSP 27 is a chaperone of the sHSP group among ubiquitin, α-crystallin, HSP 20 and others [3].

The common functions of sHSP are thermotolerance, inhibition of apoptosis, regulation of cell development and cell differentiation. Also, they have an important role in signal transduction. They can perform chaperone function by stabilizing new proteins to ensure correct folding or by helping to refold proteins that were damaged by stress [4]. HSP 27 levels can be expressed in embryonic stem cells, B-lymphoma cells, keratinocytes, neurons etc. It is possible that HSP27 plays a crucial role in the termination of growth of embryo [5].

Artificial lighting is commonly used to delay or to stimulate egg production, as the circulating levels of the luteinizing hormone (LH) and of the follicle-stimulating hormone (FSH) increase within a single day of exposure to long photoperiods [6]. It has been suggested that short wavelength light-exposed group chickens (blue light) appeared to have higher egg production due to the photo stimulation of retinal photoreceptors [7]. Otherwise, birds reared under red spectrum of light had increased level of egg formation and oviposition and laid more eggs even later in the productive period [8]. 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 of the birds than of short wavelengths (towards blue-green spectrum) leading to improvement of the reproductive performance of birds [9]. Egg number, egg weight and egg mass is dependent on the proper management of light, involving the quantity, the color and the frequency spectrum [10].

Therefore, this work was designed to study whether the LED light colour temperatures have effects on egg production parameters, and HSP 27 gene expression pattern in Fayoumi chicken layers.

2. MATERIAL AND METHODS

2.1. Experimental design

A total of 165 Fayoumi healthy pullets (17 weeks) with an average body weight of 900 grams were used in this experiment. The birds were purchased from experimental farm for poultry breeding in Fayoum governorate, Egypt. All birds were housed in the same place till laying, at a density of 10 birds/m^2. The photoperiod was 12L:12D. The relative humidity during the experiment ranged from 67 to 77 %. The average temperature in the house was 28°C. From 19th week lighting schedule was gradually increased half an hour every week till it reached 16L:8D lighting schedule at laying at 24 weeks [11]. As soon as laying started, the birds were divided into three groups in three separated, well ventilated environmentally controlled rooms according to the colour temperature of light. Each room housed 55 birds (5 males and 50 females). The first group was exposed to white LED light (6500 kelvin). The second group was exposed to blue LED light (10000 kelvin). The third group was exposed yellow LED light) (2700 kelvin) till the end of the experiment. Light intensity was 25 lx [12] and light intensity during dark periods was 0 lx. Light intensity was recorded near the floor, approximately at the bird's height. Artificial light systems were placed 10 cm above the birds using plastic crosses attached to the ceilings of the rooms. Feed intake was controlled daily according to standard farm husbandry practices to meet the nutrient recommendations for poultry of national research council [13] (Table 1). Drinking water was allowed ad-libitum throughout the experimental period (3 months from laying time).

2.1. Studied traits

I. Egg number (EN) and egg weight (EW)

EN and EW were estimated daily for each group according to [14].

II. Egg mass per hen per week (EM)

EM was equal to the number of eggs per group multiplied by the mean egg weight in grams [15].

2.2. Sample collection

Ten samples of liver (five males and five females) were taken from each group. The samples were put in Eppendorf containing RNA later (Qiagen, Germany), to minimize the action of endogenous RNase, extraction was done using RNeasy Mini Kit (Qiagen, Germany), following the manufacturer's protocol. The obtained RNA was treated with RNAse free-DNAse (Qiagen, Germany), I to remove any contaminating genomic DNA.

Table 1. Composition of the diet and the calculated analysis used throughout the experiment.

Ingredient	Laying
Yellow corn (kg)	65
Soybean meal (44% cp) (kg)	23.3
Wheat bran (kg)	1.90
Di- calcium phosphate(kg)	1.50
Limestone(kg)	7.6
Nacl (kg)	0.30
Premix (vitamins minerals mixture) (kg)	0.30
Methionine (kg)	0.10
Total (kg)	100
Calculated analysis:	
Metabolizable energy k cal /kg	2700
Crude protein%	17
C/P ratio	168
Calcium %	3.30
Available phosphate %	0.40
Lysine%	0.73
Methionine"%	0.32
Methionine and cystine%	0.62

RNA concentration and purity were 108 checked with NanoDrop® ND-1000 spectrophotometer. The cDNA of each sample was synthesized according to the manufacturer's instructions (Sensifast, Bioline, UK) (200 U/µL). The reaction mixture was performed in a total volume of 25 μL containing an equivalent of one micrograms of total RNA, 4 μL 5x Trans Amp buffer, 1 μL reverse transcriptase and DNase free-water up to 20 μL. The final reaction mixture was put in a thermal cycler and the PCR conditions were; primer annealing at 25 C^o for 10 min, reverse transcription at 50 C^o for 30 min then inactivation at 85 C^o for 5 min. The samples were kept at 4 C^o then the cDNA products were stored at -20 C^o till they were used for downstream application. Relative expression of mRNA level was carried for HSP 27. The sequence of the used primers was illustrated in table 2.

Table 2. Sequence of forward and reverse primers used in determination of the gene expression of the selected genes.

Gene	Primer (forward)	Product length (bp)	Accession number	Reference
β-Actin	F:5-GAGAAATTGTGCGTGACATCA-3 R:5-CCTGAACCTCTCATTGCCA-3	152	NM_205518.1	[16]
HSP 27	F:5-TAAGGATAACATCGTGGAGATCA-3 R:5-CTACTTCTTGGCTGGTTCTTCCT-3	250	NM_205290.1	[17]

Table 3. Cycling conditions for real time PCR.

Gene	Reverse transcription	Primary denaturation	Amplification (40 cycles)			Dissociation curve (1 cycle)		
			Secondary denaturation	Annealing (Optics on)	Extension	Secondary denaturation	Annealing	Final denaturation
ß. actin	50˚C 30 min.	94˚C 15 min.	94˚C 15 sec.	51˚C 30 sec.	72˚C 30 sec.	94˚C 1 min.	51˚C 1 min.	94˚C 1 min.
HSP27	50˚C 30 min.	94˚C 15 min.	94˚C 15 sec.	59˚C 30 sec.	72˚C 30 sec.	94˚C 1 min.	59˚C 1 min.	94˚C I min.

Table 4. Effect of light colour temperature on egg number, weight and mass of female Fayoumi chicken.

Group	Egg number	Egg weight (g)	Egg mass (g)
Control (white)	$38.66^{b} \pm 1.85$	$1631.73^{b} \pm 89.2$	$63038.52^{b} \pm 47.03$
Blue	$44.86^{a} \pm 1.53$	$1990.73^{a} \pm 86.3$	$89271.40^{a} \pm 53.32$
Yellow	$39.13^{ab} \pm 2.64$	$1685.00^{b} \pm 130.4$	$65934.05^{b} \pm 32.93$

*Means of different levels within the same column having different superscripts are significantly different ($P < 0.05$).
white: white color light exposed group.
Blue: blue color light exposed group.
Yellow: yellow color light exposed group.

2.4. Data analysis

At the end of PCR, amplification curves and CT values were determined by the stratagene MX3005P software. To estimate the variation of gene expression on the RNA of the different samples, the CT of each sample was compared with that of the control group according to the "ΔΔCt" method stated by [18].

ΔΔCT =ΔCT of treated -ΔCT of untreated.
ΔCT=CT of the target -CT value of the housekeeping gene.

Data of egg number, egg weight and egg mass were expressed as means ± standard error of the mean. Comparisons were performed using General Linear Model (GLM) procedures of SPSS 4 package for repeated measures (SPSS, 1999). Duncan Multiple Range Test was used to separate the means among the treatment groups. Differences were considered to be significant at the level of (P≤0.05) [19].

3. RESULTS

Data in Table 3 show egg number, weight and mass of female Fayoumi chicken. Data showed that there was a significant difference in egg number between the blue and white colour light exposed groups and the highest value was for blue colour light exposed group and the lowest value was for white colour light exposed group. A significant difference was found in egg weight and mass between blue and both yellow and white colour light exposed groups with the highest value was for the blue colour light-exposed group and the lowest value was for the control group.

Heat shock protein 27 (HSP 27) gene expression

Data in Figure 1 represent HSP 27 gene expression in the liver of males. The data showed that there was a significant upregulation in the blue colour light-exposed group when compared to both yellow and white colour light-exposed (control) groups.

Data in Figure 2 show HSP 27 gene expression in the liver of females. The data showed that there was a significant upregulation in the blue colour light-exposed group when compared to both yellow and white colour light-exposed group.

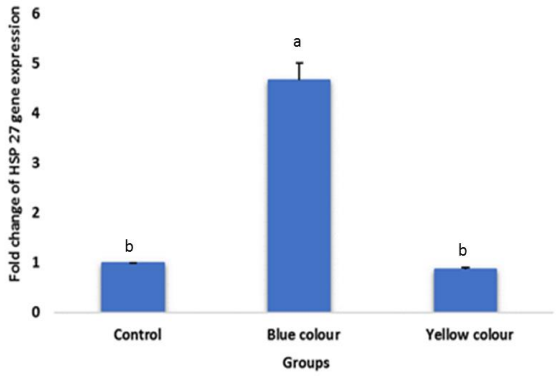

Figure 1. Means ±SE of HSP 27 gene expression pattern in the liver of male Fayoumi chicken. Small alphabetic letters show significance when (P<0.05)
HSP 27: Heat shock protein 27.
White.white color light exposed group.
Blue: blue color light exposed group.
Yellow: yellow color light exposed group.

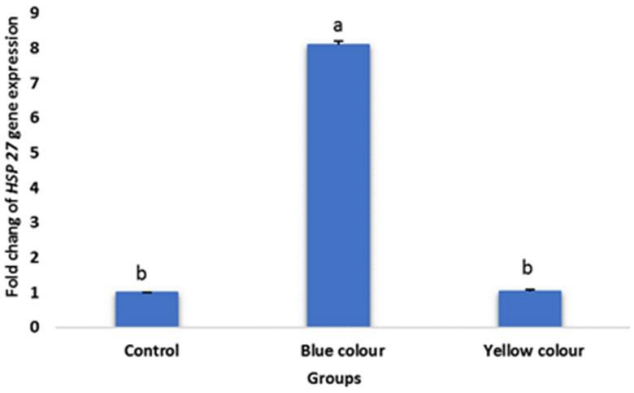

Figure 2. Means ±SE of HSP 27 gene expression pattern in the liver of female Fayoumi chicken**.** Small alphabetic letters show significance when (P<0.05).

4. DISCUSSION

This study sought to extend our overall understanding of the effects of the different light colour temperatures that are becoming available to poultry industry. For the purpose of testing new management practices, this study compared three types of commercially available LED bulbs marketed for poultry production. Poultry industry is expanding due to many socio-economic factors. Poultry production is also an important factor of agriculture all over the world. Recently, more attention has been given to poultry in particular, due to the quality of their meat and sustainable production **[20].** Layers have been submitted to genetic improvement to produce more eggs at a lighter body weight with lower feed intake. So, egg operations need to face the challenges of supplying the high nutritional requirements of layers and of designing management practices adapted to the increasingly automated and environmentally controlled facilities and to high stocking densities [21]. Light emitting diode (LED) system is a new technology which is used all over the world for increasing egg production from laying hens [22]. Egg production was maximized by using the once innovation programmed lighting. This lighting program showed a statistically significant increase of 38 eggs per hen over a 27-week production period [10]. It has been shown that monochromatic blue and green light improved welfare and performance of broiler due to stimulation of testosterone secretion and myofiber growth [23]. Immunity can also be enhanced at the later stage of rearing by subjecting birds to shorter wavelength LEDs such as blue light due to the increased circulating levels of IgG and IgA antibodies [24].

A number of studies investigated the effect of light on reproductive performance of poultry; some reported a positive impact whereas, others reported a non-significant difference. In this study, the highest egg number, egg weight and egg mass were observed in blue colour light-exposed group as blue colour light caused an upregulation of GnRH from the hypothalamus which in turn triggered FSH and LH hormone from the pituitary gland which might have increased egg production process. The results of [25] are in the same line with those reported in the current study who found that egg weight was lower in red light compared to blue and incandescent light. The finding of this context agrees with those obtained by [12] who declared that birds housed under blue light showed the highest egg production. This may have resulted from the photo stimulation of retinal photoreceptors, which are sensitive to blue light-green light which are important for endocrine glands that secrete hormones directly into the blood. This was further confirmed in a follow up study in which broiler breeder hens were exposed to green light and showed a significantly lower cumulative number of eggs and overall production levels than birds exposed to white light [26]. Similarly, It has been summarized that the short wavelength (blue light) produced a greater total number of eggs than did the long wavelength (red light) group, and this could be due to that lower melatonin increases plasma FSH, LH, progesterone, and estrogen, and decreases plasma prolactin [7]. The increase in FSH, LH and progesterone and estrogen would in turn improve egg production.

These results disagree with the results of [27] who observed no differences in reproductive performance of hens exposed to incandescent (IL) and blue light when lighting intensity remained constant. It was also reported that laying hens exposed to light sources containing higher wavelengths (red colour) produced significantly more eggs than hens exposed to light sources with lower wavelengths (green, blue) during the first laying cycle [28]. This may be due to that red light resulted in higher estradiol concentrations due to photo stimulation, causing a release of GnRH which stimulates the release of gonadotropins (follicle stimulating hormone, FSH, and luteinizing hormone,

LH) from the anterior pituitary gland, which in turn triggers gonadal development and the synthesis of steroid hormones (progesterone from the granulosa cells of the large follicles and estradiol from the small follicles), Also, It was reported that egg weight usually depends particularly on hen's age and on nutritional factors rather than on light colour [29]. Laying hens maintained under monochromatic red light had higher egg production than birds maintained under white, green [30] and blue LED light [31]. The significantly highest hen egg production was observed in laying hens reared in red light [22] and white light [29].

Additionally, It has been illustrated that different light sources did not cause a significant difference in egg production, egg weight, egg mass and egg quality parameters ($P > 0.05$) [32]. It has also been indicated that egg production was similar in white, green, and blue light colour [33]. Red LED light may increase egg weight [34] and egg production [35] due to increasing ovary stroma and ovarian follicle numbers [33]. One reason for the controversy between the results reported in the current experiment and those of the previous work could be due to differences in the light source, species/strain of the birds and the light intensity. For example, considering the effect of light source, birds under monochromatic red light had significantly higher egg production than birds maintained on green and blue light and this may be due to higher steroid and gonadotropin concentrations and higher neuropeptide mRNA expression [36].

Several investigations have reported that stress increases the synthesis of the heat shock proteins *HSP 27*, *HSP 70*, and *HSP 90*, which are also constitutively expressed and play an essential protective role in maintaining the metabolic and structural integrity of the organ against stress-induced tissue injuries [37]. In this study, it was found that the blue LED colour light-exposed group had the highest upregulation of *HSP 27* gene expression. This finding is in the same line with those of [38] who found that blue LED light caused a significant upregulation of *HSP70* and *HSP90* gene expression ($P < 0.05$) in heat stressed broiler chicken. This could be attributed to the fact that Heat shock proteins (HSPs) play an essential role in protecting and repairing cells and tissues against stress. HSPs regulate protein processing in the cells and enhance refolding of the damaged protein [39]. Furthermore, It has been highlighted that under stress conditions the stress genes related to cell survival are turned on, while less essential genes may be turned off [40]. These results are also in accordance with the findings of [41] who found that *HSP 90* gene expression increased in the heart, liver, kidney, pancreas and the pituitary gland of Shaoxing ducks under acute heat stress, and the increase was limited to the heart, liver, and pituitary after chronic exposure to heat stress. In the same line, the expression of *HSP27, HSP70*, and *HSP 90* mRNA in the bursa of Fabricius and spleen were upregulated ($P < 0.01$) in response to heat stress, but those of *HSP 27* and *HSP 90* mRNA in thymus were downregulated ($P < 0.01$) in black boned chicken [17]. Also, during heat stress higher HSP expressions could be observed in almost all body organs of chicken [42]. This may be due to the fact that HSP act as a cellular mechanism to protect different cells against damage. Therefore, the blue LED light could be recommended for use in laying farms as it enhanced reproductive parameters of birds and improved ability of birds to cope with the environment when compared to other light colour temperatures.

Conclusion

Blue LED light improved the bird reproductive performance and upregulated the HSP 27 gene expression.

Conflict of interest statement

The authors declare that there is no any conflict of interest in the current research work

Research ethics committee permission

The current research work was executed according to standards of Research Ethics Committee, Faculty of Veterinary Medicine, Mansoura University.

Authors' contribution

Huda A. EL- Emam conducted the experiment and analytical procedures; Ahmed I. Ateya helped with writing the manuscript; Iman E. EL- Araby performed statistical analysis and helped in writing the manuscript; Usama A. Abou-Ismail revised the manuscript; Mohamed M. Fouda edited the manuscript.

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