Biotechnology in Animal Husbandry 30 (3), p 457-471 , 2014 ISSN 1450-9156
Publisher: Institute for Animal Husbandry, Belgrade-Zemun UDC 636.083.31'636.5
DOI: 10.2298/BAH1403457M

REARING BROILER CHICKENS UNDER MONOCHROMATIC BLUE LIGHT IMPROVE PERFORMANCE AND REDUCE FEAR AND STRESS DURING PRE-SLAUGHTER HANDLING AND TRANSPORTATION

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Original scientific paper

Abstract: The aim of this study was to evaluate the effect of monochromatic light on broiler performance, fear and stress response during pre-slaughter handling and transportation. Two thousand unsexed one-day old Cobb broiler chicks were used. At day 34, two hundreds broilers of similar live body weight were selected and divided into two equal groups (2 group x 5 replicates). Broilers were reared under white light (WL) from 0-34 day. From 35 to 49 day, the first group was reared under WL and the second group under blue light (BL). Final body weight (FBW), tonic immobility reactions (TI), respiratory rate (RR), heterophils to lymphocytes (H/L) ratio and interlukien-1β (IL-1β) were estimated at day 49 before and after transportation. After transportation, weight of internal organs (liver, spleen, heart and bursa of fabricius) as a percentage of FBW was calculated. Results showed that there was a significant ($P < 0.05$) increase in FBW and reduced weight loss due to transportation in broilers reared under BL. In broilers reared under BL: TI duration, RR, H/L ratio, IL-1β and weight of internal organs (except the heart) were significantly ($P < 0.05$) lower. The interaction effect of light and transportation on TI duration, RR, lymphocytes, H/L ratio and IL-1β were significant ($P < 0.05$). Therefore, it is suggested that BL may be a good tool for improving welfare and mitigating stress not only in pre-slaughter handling but also during transportation of broilers.

Key words: Broilers welfare, monochromatic BL, transportation, tonic immobility, H/L ratio

Introduction

In modern poultry husbandry, light has become an important factor that can be used to improve broiler welfare. Light color has been considered a powerful management tool that can be used for mitigating several stressors in broilers by modulating many physiological, immunological and behavioral pathways *(Xie et al, 2008 and 2011; Lewis and Morris, 1998).* It has also been found that broiler performance could be affected by the light spectra *(Rozenboim et al., 1999; Halevy et al., 1998)*. Broilers reared under blue or green light were significantly heavier than those reared under red or white light *(Rozenboim et al., 2004)*. Several studies indicate that blue light (BL) which characterized by short wavelength seems to stimulate broiler growth at the end of the production cycle (27-49 day) without significant effects on total feed consumption, food conversion ratio and/or mortality rate *(Halevy et al., 1998; Rozenboim et al., 1999; 2004; Cao et al., 2008; Ke et al., 2011)*. It also has an important role in reducing stress, decreasing fear, modulating the stress response and has been suggested to have a calming effect on broilers (*Prayitno et al., 1997; Glatz 2005; Mohamed 2011; Xie et al,. 2008).*

Broilers housed under intensive management systems are subjected to a lower degree of human contact, particularly at systems in which environmental conditions and provision of feed and water are automated. This may produce a fear of humans *(Zulkifli et al., 2002)*. It is well documented that procedures with high human contact such as catching and crating induce stress and fear reactions. At the end of the production cycle, broilers are harvested and transported to the slaughter plant for slaughtering. Several studies have been conducted to determine which stage of the pre-slaughter processes of transportation is the most stressful event for broilers. During harvesting and transportation, broilers are subjected to several stressors such as feed and water deprivation, physical contact with workers, social disruption, noise, overcrowding, vibration and thermal extremes *(Mench, 1992; Mitchell and Kettlewell, 1998; Delezie et al., 2007)*. It has also been found that improper handling and transportation of broilers may result in injuries, increase fear and decrease immune responses, resulting in high mortalities *(Savenije et al., 2002; Nijdam et al., 2004 and 2005; Vieira et al., 2010; Knowles and Broom, 1990). Cashman et al. (1989)* reported that fear levels in birds were mainly determined by transportation and not just by catching and loading.

Many studies have been conducted to determine the effect of light on broiler performance, and many others on the effect of transportation. However, there has been a very few investigation on the influence of light color on transportation of broilers. The objectives of this study was to determine the effect of monochromatic light on broiler performance and to evaluate its role in reducing fear and stress in broiler during pre-slaughter handling and transportation.

Materials and methods

Ethical approval

Animal ethics committee, Faculty of Veterinary Medicine, Kafrelsheikh University, Egypt, approved the protocol and conduct of the study.

Poultry housing and management

Two thousands unsexed one-day-old Cobb broiler chicks were used in this study. From day 0-34, all birds were reared in the same pen at a stocking density of 11 birds/m^2. The floor was concrete with wood shavings as a bedding material. Birds had ad libitum access to feed and water. Ration was formulated to meet the nutrient recommendations for broilers by the National Research Council *(NRC, 1994)*. At day 34, two hundreds birds of similar live body weight were selected and divided into two equal groups. From day 35-49, each group was reared in a separate pen divided into 5 equal replicates kept under the same conditions, except for the color of light.

Light treatment

All birds were reared under WL from zero to 34 day. From 35-49 day, two different types of light were used; the first group was reared under WL (400 to 700 nm) and the second one was reared under BL (480 nm). The light schedule was constant at 23L: 1D under 15 lx light intensity during the entire experiment. The duration of natural light was 10 h and 56 m, 10 h and 15 m and 10 h and 7m at day 0, 34 and 49 respectively then followed by artificial light either white or blue. The dark hour was set at 21.00: 22.00 h.

Handling and transporting conditions

Feed was withdrawn 6 h prior to birds harvesting. Water was available for ad libitum consumption until 1 h before the birds were manually caught and crated. Individual birds were picked up in an upright position with both hands and placed in the crates (100 x 50 x 25 cm) at a density of 10 birds/crate. Birds were handled as gently as possible in order to avoid physical damage or stress. The duration of transportation was 5 h with an average speed of 60 ± 5 km/h. The average environmental (external) temperature was 23 ± 1.2 °C and core (internal) temperature of 33 ± 2.7 °C.

Blood sampling and analysis

Blood samples (3mL/bird) were aspirated from the wing vein and transferred into vacuum tubes with or without ethylenediaminetetraacetic acid (EDTA). The whole blood (with EDTA) was used for heterophils (H) and lymphocytes (L) count and calculation of heterophils to lymphocytes (H/L) ratio. Serum samples were separated by centrifugation of blood at 3000 rpm for 10 m and were stored at −20°C until analysis.

Experimental treatments

1. Broiler performance

Chicks were individually weighed at day 0, 34, 49 before and after transport (using Sartorius balance produced by Sartorius– universal, made in Germany). Individual live body weights were totaled and divided by the number of chicks to obtain the average live body weight (LBW). The final body weight (FBW) was recorded at day 49 after handling and directly after transportation for 25 randomly selected birds from each light treatment.

2. Tonic immobility (TI) reactions

Immediately following the handling and directly after transportation and unloading of broilers, 25 randomly selected birds from each light treatment were tested individually for the duration of TI. The birds were carried to a separate room (no visual contact with other birds) and subjected to TI measurements. TI was induced as soon as the birds were carried to a separate room by gently restraining the bird for 15 s on its right side by the legs and wings. The researcher then remained observing the bird without unnecessary noise or movement. Direct eye contact between the observer and the bird was avoided as it may prolong TI duration *(Jones, 1986).* A stopwatch was used to record latencies until the bird righted itself. If the bird righted itself in less than 10 s, the process was repeated. If TI was not induced after three attempts, the duration of TI was considered as 0 s. The maximum duration of TI allowed was 600 s *(Campo and Carnicer, 1993)*. The number of inductions required to perform TI were also recorded.

3. Respiratory rate (RR)

Respiratory rate was recorded for 25 randomly selected birds by counting the number of thoracic movement visually *(Kassim and Norziha, 1995)* before and after transportation for each light treatment.

4. Heterophils/ lymphocytes ratio (H/L ratio)

Heterophils, lymphocytes and H/L ratio in whole blood were measured using an automatic blood cell counter (exigo-Vet., BOULE MEDICAL AB Inc., Stockholm - Sweden.) after handling and immediately after transportation and unloading processes for 25 birds from each light treatment.

5. Serum IL-1β

The serum IL-1β of 25 birds from each light treatment was measured using a commercial broiler ELISA kit (BioSource International Inc., Beijing, China) before and after transportation *(Xie et a,. 2008).*

6. Weight of internal organs

Immediately after transportation and unloading processes, 10 birds of an average body weight from each light treatment were carefully euthanized via exsanguination from a neck cut that severed the carotid artery and jugular vein. This method is considered humane when performed by a trained person (Gracey 1986). The birds were eviscerated to harvest the liver, spleen, heart and bursa of Fabricius. The organs were gently soaked in 0.9% ice-cold saline to remove the remaining blood. Harvested organs were immediately weighted by digital balance (PW Balance, ADAM equipments Co., USA).

Statistical Analysis

Data were tested for distribution normality and homogeneity of variance. Data were reported as means ± SEM and analyzed by ANOVA with Minitab software version 16. Differences in parameters between groups were compared with Student-t test. The significance level was set at $P < 0.05$.

Results

Data analysis revealed that, there were no significant differences of all measured parameters between the five replicates within the experimental groups.

Broiler performance

Body weights (g) of chicks on day 0 were 40.72 ± 2.14 (minimum) and 47.42 ±1.09 (maximum). On day 34, mean body weight (g) of WL group was 1562.92 ± 53.24 and BL group was 1560.67 ± 35.73. The results for FBW are shown in table 1. Birds reared under BL had a higher FBW ($P < 0.05$) and lost significantly less body weight during transportation and unloading ($P > 0.05$) than those reared under WL.

Table 1. Effect of light treatment and transportation on final body weight, tonic immobility (TI) induction, TI duration, respiratory rate (RR), heterophils, lymphocytes, heterophil to lymphocyte (H/L) ratio and interlukien-1β (IL-1β) of broiler.

Variable	Before transportation		After transportation		P- value		
	WL	BL	WL	BL	light	Transport	Interaction
Final body weight (kg)	2.189 ± 0.018	2.372 ± 0.031	2.143 ± 0.017	2.336 ±0.025	0.0001	0.086	0.835
TI induction	1.480 ± 0.154	1.720 ±0.147	1.120 ±0.167	1.280 ±0.147	0.197	0.011	0.796
TI duration (s)	263.44 ± 1.42	224.60 ±1.26	462.24 ±2.36	340 ±4.09	0.0001	0.0001	0.0001
RR/ min	55.160 ±0.639	47.080 ±0.622	67.840 ±0.607	56.360 ±0.458	0.0001	0.0001	0.005
Heterophils (per 100 cell)	18.440 ±0.259	13.200 ±0.337	19.120 ±0.362	15.120 ±0.291	0.0001	0.0001	0.052
Lymphocytes (per 100 cell)	38.760 ±0.456	41.320 ±0.446	40.080 ±0.412	39.840 ±0.320	0.006	0.846	0.001
H/L ratio	0.477 ±0.009	0.320 ±0.008	0.478 ±0.011	0.380 ± 0.008	0.0001	0.001	0.001
IL-1β (pg/mL)	15.936 ±0.391	2.032 ± 0.082	36.340 ±0.477	8.575 ±0.280	0.0001	0.001	0.0001

The significance level was set at P < 0.05 within the same row.

Fear and stress indices

Analysis of TI duration (table 1) revealed significant differences between birds reared under BL and WL before and after transportation. The interaction effect of light and transportation (Fig. 1) was significant (P < 0.05). There was no significant (P > 0.05) effect of light on number of TI inductions, while transportation had a significant (P < 0.05) effect on TI induction number.

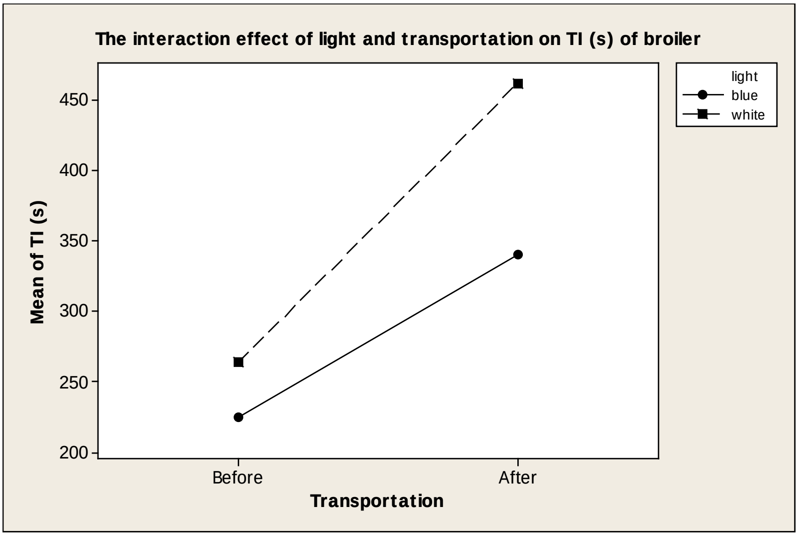

Figure 1.The interaction effect of light and transportation on TI duration(s) of broiler

Results in table one indicated that the RR/min was higher in birds reared under WL than BL before and after transportation. There were significant (P < 0.05) effects of light and transportation on RR/min, and the interaction between the two factors (Fig. 2).

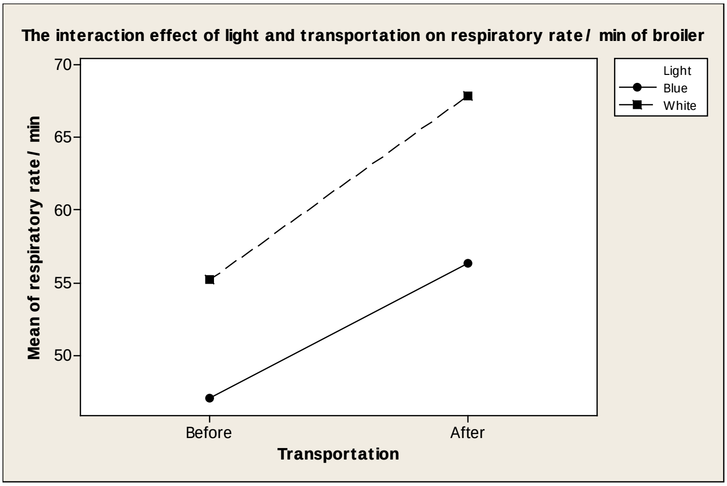

Figure 2.The interaction effect of light and transportation on respiratory rate (RR)/ min of broiler

Heterophils (table 1) was higher in broilers exposed to WL than those exposed to BL before and after transportation. The light colour and transportation had a significant (P < 0.05) effect on heterophils but the interaction effect was not significant.

There was a significant (P < 0.05) effect of light colour on lymphocytes (table 1). While the effect of transportation on lymphocytes was not significant and the interaction between light and transportation was significant (Fig.3).

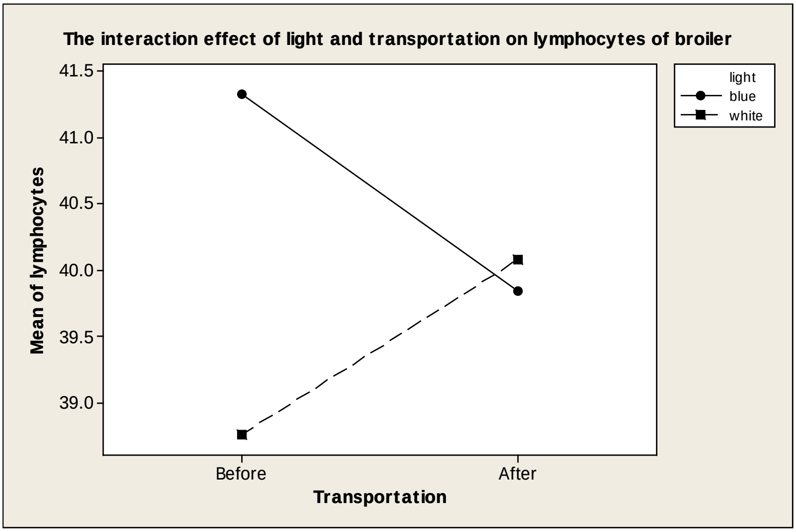

Figure 3.The interaction effect of light and transportation on lymphocytes of broiler

Broilers exposed to WL had significantly (P < 0.05) higher H/L ratios than those exposed to BL before and after transportation (table 1). The interaction effect of light and transportation was also significant (Fig. 4).

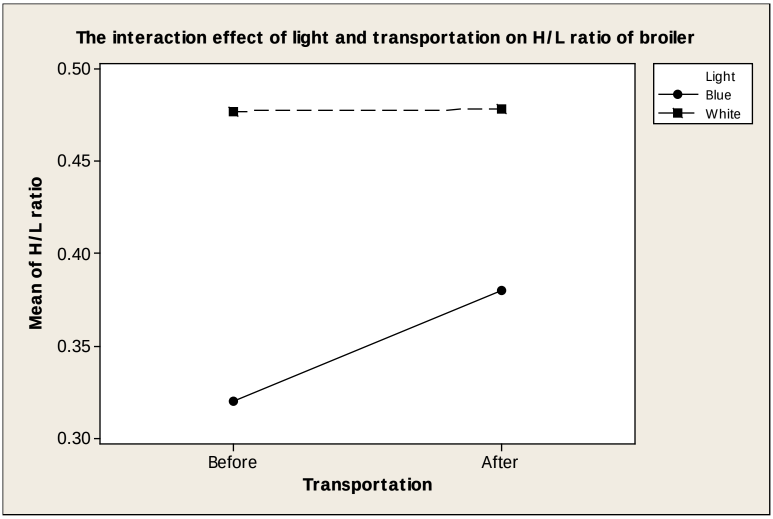

Figure 4.The interaction effect of light and transportation on H/L ratio of broiler

Serum samples of IL-1β in table one were significantly higher (P < 0.05) in broilers reared under WL than those reared under BL before and after transportation. The interaction effect of light and transportation on IL-1β was significant (Fig. 5).

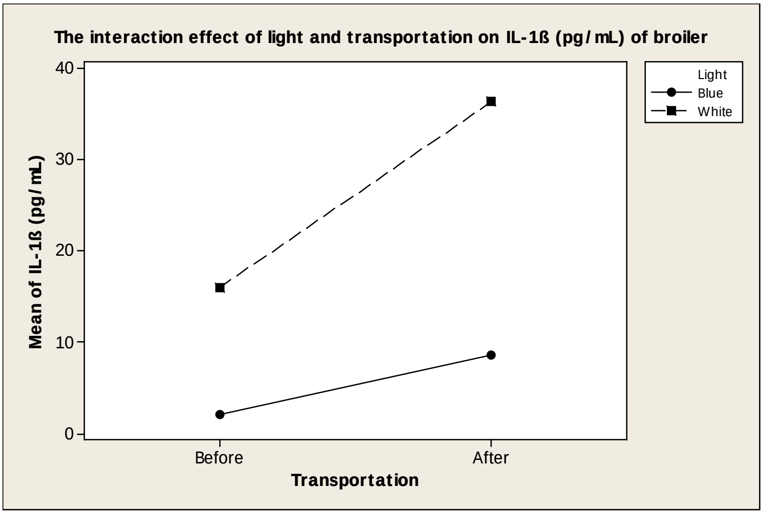

Figure 5.The interaction effect of light and transportation on interlukien-1β (IL-1β) of broiler

After the transportation and unloading processes, the weight of the liver, spleen and bursa of Fabricius in broilers reared under WL was significantly higher (P < 0.05) than BL (table 2). No significant difference between light treatments was detected in heart weight.

Table 2. Effect of light colour on weight of internal organs (to final body weight, %) of broiler chickens after transportation

Variable	WL	BL
Liver	2.9460 ± 0.0428[a]	2.1700 ± 0.0610[b]
Spleen	0.2008 ± 0.0216[a]	0.1152 ± 0.0099[b]
Heart	0.7118 ± 0.0660[a]	0.5374 ± 0.0276[a]
Bursa of Fabricius	0.09232 ± 0.00705[a]	0.05376 ± 0.00235[b]

Mean values within the same row with different superscript are significantly different (P<0.05).

Discussion

In this study, the effects of monochromatic light on growth performance and in mitigating stress during handling and transportation of broilers were investigated. The welfare of broilers was assessed via performance, TI, RR, H/L ratio, IL-1β and weight of internal organs. The results showed that the spectrum of monochromatic BL positively affects the growth rate of broilers; rearing of broilers under BL from 35-49 d was found to increase the FBW. Our results are in agreement with the results from previous work by *Wabeck and Skoglund (1974); Prayitno (1994); Celen and Testik (1994); Rozenboim et al., (2004); and Cao et al., (2008)*. This enhancement in growth may be due to the elevation of plasma androgens that increase protein synthesis and decrease destruction, consequently maintaining myofibrils and muscle growth *(Bates et al., 1987; Salomon et al., 1990; Crowley and Matt, 1996; Rozenboim et al., 1999; Cao et al., 2008)*. Monochromatic BL may also improve the quality and antioxidation of muscles, which improves FBW of broilers at later stages of growth *(Ke et al., 2011)*.

Body weight loss after transportation in birds reared under BL was lower than those reared under WL. This may be due to the birds being less active, calm and having a well-developed small intestine that indicates good feed absorption (*May et al. 1990; Prayitno et al., 1997; Buhr et al., 1998; Xie et al., 2011*).

Tonic immobility duration in broilers reared under BL was lower than broilers reared under WL. This may be due to the calming effect of BL, causing the birds to become less active and less nervous *(Prayitno et al. 1997)*. BL provides adequate illumination for workers but not for broilers, and consequently reduces the movement and escape behavior while harvesting the broilers *(Gregory et al. 1993).*

The results showed that RR/min was increased immediately after handling, loading and during transportation and unloading of broilers reared under WL. However, RR was reduced in broilers reared under BL. This may be due to the exposure of broilers to high temperature, overcrowding and vibration during transportation *(Freeman 1984; Minka and Ayo 2007)*. The previous factors may be mitigated in birds reared under BL through its calming effect.

Heterophils to lymphocytes ratio is used as an indicator of stress. Some previous studies *(Mahmoud and Yaseen 2005; Dozier et al., 2006; Mumma et al., 2006; Al -Aqil and Zulkifli, 2009; Kang et al., 2011)* reported that H/L ratio increased under the stressful conditions. In the present experiment, broilers reared under BL had lower H/L ratio than broilers reared under WL before and after transportation. This may be attributed to the calming effect of BL and its effect on modulation of stress response in broilers *(Prayitno et al., 1997; Xie et al., 2008)* and the birds become less aggressive and less active *(Glatz 2005; Mohamed 2011).*

Concentration of IL-1β in serum has been used to demonstrate the effect of monochromatic light on the stress response in broilers in many studies. The IL-1β can stimulate specific hypothalamic neurons to secrete corticotrophin releasing hormone, which stimulates the adrenal cortex to secrete corticosterone *(Berkenbosch et al., 1987)*. Corticosterone is one of the most reliable indicators of stress in broilers, especially during handling and transportation *(Kannan et al.*

1997; Nijdam et al., 2005; Zhang et al., 2009). Therefore, IL-1β could be considered an indicator of the stress response in birds. In our study, rearing broilers under monochromatic BL has reduced the level of serum IL-1β both after handling and directly after transportation. Serum IL-1β levels were greater in the broilers reared under WL than BL. These results and results from other studies *(Xie et al., 2008)* indicate that BL may be helpful to prevent excessive IL-1β expression compared with WL. In addition, the results demonstrate that exposure of birds to BL (35-49 d) can reduce the adverse effects of stress during pre-slaughter handling and transportation.

There were significant differences in the weight of liver, spleen and bursa of Fabricius of broilers reared under WL and BL after transportation. These results indicated that BL reduced the weight of internal organs. This could be due to the reduction of stress and improvement of general health *(El-Saidy, 2011).*

Conclusion

Monochromatic BL improve broilers performance, welfare and reduce fear and stress during pre-slaughter handling and transportation. Our study recommends that the catching of broilers should be carried out under BL to calm the birds. Further studies are required to make additional development in this area of science.

Acknowledgments

The authors are grateful to the Central Diagnostic and Research Laboratory, Faculty of Veterinary Medicine, Kafrelsheikh University, Egypt for helping us in the laboratory work.

Uticaj uzgoja brojlera pod monohromatski plavim svetlom na poboljšanje performansi i smanjenje stresa tokom transporta i postupka pred klanje

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Rezime

Cilj ovog istraživanja je bio da se proceni uticaj monohromatske svetlosti na performanse brojlera, strah i stres kao odgovor na rukovanje pticama tokom transporta i pre klanja. Dve hiljade jednodnevnih Cobb brojlerski pilići, oba pola, su korišćena u istraživanju. U uzrastu od 34 dana, 200 brojlera slične telesne mase su izabrani i podeljeni u dve jednake grupe (2 grupe x 5 ponavljanja). Brojleri su gajeni u uslovima sa belom svetolosti (WL) od 0-34 dana. Od 35. do 49. dana, prva grupa je gajnae pod WL a druga grupa pod plavom svetlošću (BL). Završne telesne mase (FBV), tonične reakcije (TI), respiratorna stopa (RR), odnos heterofila i limfocita (H/L) i interlukien-1β (IL-1β) su procenjeni 49. dana, pre i posle transporta. Posle prevoza, težina unutrašnjih organa (jetre, slezine, srce i burza - *Bursa fabricii*) je izračunata kao procenat FBV. Rezultati su pokazali da postoji značajno (p <0,05) povećanje FBV i smanjenje gubitka težine zbog transporta kod brojlera gajenih pod BL. Kod brojlera gajenih pod BL: trajanje TI, RR, H/L odnos, IL-1β i težine unutrašnjih organa (osim srca) bili su značajno (P<0,05) niži. Interakcija efekta svetlosti i transporta na trajanja TI, RR, limfocite, H/L odnos i IL-1β je bila značajna (p<0,05). Stoga se sugeriše da je BL možda dobar alat za poboljšanje dobrobiti i ublažavanje stresa, ne samo pred klanje, već i tokom transporta brojlera.

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Received 2 June 2014; accepted for publication 22 September 2014