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Effect of different light colors on behavior and fear reactions of Japanese Quails (*Coturnix coturnix japonica*)

Safinaz A. Ibrahim[1], Radi A. Mohamed[2]

[1]Department of Animal Husbandry and Animal Wealth Development, Faculty of Veterinary Medicine, Damanhour University (22511), **Egypt**

[2]Department of Aquaculture, Faculty of Aquatic and Fisheries Sciences, Kafrelsheikh University, Kafr El-Sheikh (33516), **Egypt**

***Correspondence: safiibi1985@yahoo.com** **Received: 14 Nov. 2019, Revised: 19 Dec. 2019 Accepted: 20 Dec. 2019 e-Published: 26 Dec. 2019, Reviewed by: Prof. Dr. Hamada D.H. Mahboub, Prof. Dr. Sameh Gad**

Important environmental cues are light frequency, duration and wavelength that affect behavior and fear response of quails. The purpose of this study was therefore to assess the impact of different light colors on the behavior and fear of quails. One hundred and fifty Japanese quails (6-weeks old), were arbitrarily allocated to 5 treatments according to light color (white, green, blue, yellow and red) and each treatment was subdivided into 3replicate each. The results revealed that, there was no significant difference in the behavior of quails except for drinking and sexual activity between light treatments. However, rearing Japanese quail under white light increased percentage of feeding, standing, movement activities, wing and leg stretch and shaking behaviors, while crouching, wing stretch and preening behaviors tended to be higher in quails received yellow light. Moreover, red light increased agonistic behavior of quail. Green light group had higher pecking, huddling behaviors. Ruffling behavior and sexual display observed to be at highest level under blue light. The longest tonic immobility duration was recorded in white light followed by green light, yellow light and red light. However the shortest duration was recorded in quails reared under blue light. The red light showed the most successful mating activity and blue light showed the best sexual display. It was concluded that rearing quails under blue light is preferable due to its soothing effect on bird's more than the other light colors, although red light has the best photosexual stimulatory effect than the other light colors.

Keywords: light color, Japanese quails, behavior, tonic immobility

INTRODUCTION

Japanese quail (*Coturnix coturnix japonica*) is characterized by rapid growth, low feed intake, early sexual maturation and high egg production. In addition, it has high resistance to most of common diseases affecting other types of domestic birds. Furthermore, it could be reared under adverse environmental and climatic conditions (Nunes et al., 2016).

Light duration, intensity, and color have been known as major environmental stimuli that affect physiology, behavior, immunity, and growth performance of birds (Olanrewaju et al., 2016). Several types of lighting systems have been used in the poultry production houses including fluorescent, compact fluorescent and incandescent lighting systems.

Light is an environmental factor affecting quail production, physiology, reproduction as well as behavioral parameters of the birds (Parvin et al., 2014). In addition, artificial light colors are important environmental factors controlling growth performance and reproductive activity of Japanese quails (Elkomy et al., 2019). The implementation of appropriate lighting programs is important for the production of eggs because the light stimulus directly affects the bird's physiological responses, thus stimulating the release of reproductive hormones, accelerating or delaying sexual maturation and stimulating the laying of eggs (Freitas et al., 2005).

Yet, it is become difficult to choose the best method of lighting to attain the highest growth performance, physiological responses of quails. Color of light affects production differently throughout the stages of bird's production (Olanrewaju *et al.,* 2016; Mohamed *et al.,* 2014; Mohamed et al., 2017a; Mohamed et al., 2017b). Some reports from studies of modern birds production suggest that light color can affect birds behavior, with blue light with a soothing influence, red light decreases the pecking of feather and cannibalism, and blue-green single or mixed LED light stimulating growth performance (Rozenboim et al., 2004; Kim et al., 2013). Moreover, Mohamed *et al.,* (2015) found that rearing Japanese quail under blue light ameliorate the effect of heat stress and the birds become calmer and reduce fear reaction. In addition, Sabuncuoğlu et al., (2018) found that the blue and green light applied to Japanese quail eggs in incubation did not affect the characteristics of incubation, development and carcass slaughter, but had positive effects on certain characteristics of behavior. Light colors, on the other hand, influence bird reproduction (Nunes et al., 2016). Bobadilla-Mendez et al., (2016) reported that white light photostimulation was more successful in triggering the reproductive cycle, promoting the initiation of sexual maturity and through the post-puberty growth of reproductive organs. Although red light can be used to substitute fluorescent lamps by encouraging the same live performance, egg quality and morphological growth of the laying Japanese quail reproductive tract (Nunes et al., 2016). In addition, Elkomy et al., (2019) concluded that using red light color during the period of growth and laying in Japanese quails improves growth and reproduction performances. Yadav and Chaturvedi, (2015) proposed that long-term exposure to low-intensity blue LED light could cause gonadal regression even under long-term conditions (L: D= 16:8), whereas exposure to green and red light tends to maintain constant photosensitivity after a maximum gonadal period. From the previous literatures, there was a controversy over the influence of light color on the behavior and reproduction of Japanese quail. The purpose of this research was therefore to assess the impact of different light colors on Japanese quails ' behavior and fear response.

MATERIALS AND METHODS

2.1. Bird management and experimental design

In a controlled environment, a replication study was carried out using a total of one hundred and fifty 6-weeks old of mixed sex Japanese quails. They obtained from poultry and rabbit project, Department of Animal Husbandry, Faculty of Veterinary Medicine, Alexandria University. Quails were equally allocated to five light- treated groups on deep floor system of rearing, which covered with 10-12 cm of clean, dry and soft wood shaving after sprinkling slaked lime over the floor of pens. Dimensions of pens were (100cm length x 100cm width ×100 cm height) / 30 birds separated from each other by plywood partitions. Each treatment subdivided into 3 replicates (10 birds each).They illuminated with 60 watt colored reflector lamps (White, Yellow, Blue, Red and Green) installed at the top of each pen, the birds maintained on continuous lighting system (24L: 0 D) throughout the trial in the lighted chamber (Omer Coban et al., 2009). Temperature was adjusted at the room temperature (25-28 ˚Ċ). The quails had access to feed and water for ad-libitum, and they were fed on commercial ration contain 18% protein (El-Kahera Company).

2.2. Behavioral observation

Observation carried out by Scanning instantaneous observations according to Martin and Frs, (1986), from 6th week till 12th week of rearing, four times per day, at early morning (7:00-8:00 a.m.), late morning (10:00-11:00 a.m.), early afternoon (1:00-2:00 p.m.) and late afternoon (4:00-5:00p.m.) for three days weekly. For the following behaviors, observations have been recorded: ingestive behaviors (eating and drinking), resting behavior (crouching and huddling), comfort behavior (stretching both wings, wing and leg stretching, ruffling and shaking), maintenance behavior (preening), inactive behavior (standing), exploratory behaviors (feather pecking, trough pecking and litter pecking), Movement behavior (walking and running), agonistic behavior (chasing and aggressive pecking) and sexual display. It was known that eating and drinking had stopped as soon as the bird became quiet, even if it was in front of a feeder or a drinker. Crouching behavior meant lying or sitting of bird, breast on the floor, looking about or with closed eyes, no other behavior. Huddling behavior meant three or more birds were overlapped a crouching position in an allelomimetic pattern. A quail was deemed to be walking only when the bird moved and did not engage in any other activity; short periods of immobility were deemed to be standing or standing without any other activity. Quails pecking at any object in addition to a feeder or drinker were considered to be pecking an object. Preening was taken into account when manipulating plumage with the beak. Stretching behavior included wing stretching, leg/ wing, and both legs stretching. Exploration ordered as: a- Trough exploration: pecking at trough, usually with intermittent scratching. b- Wall exploration: pecking at inedible objects like wall of the pen. c- Feather pecking: pecking or preening like acts directed to another birds feathers. Moreover, Sexual behaviors included: a- Incomplete mating: In which the male grasps the back of the head of the female and tries to mount without vent placement or ejaculation. b- Complete mating: in which male grips the back of the head of the female, assembly attempts, vent placement, ejaculation and disassembly. 3- Crowing: the voice produced by the male at the starting of sexual behavior.

2.3. Behavioral screening and acquisition of the social proximity response:

Male sexual behavior in Japanese quail includes appetitive and consummatory component (Balthazart and Ball, 1998). Some stereotyped behaviors result in a functional outcome that is associated with a reduction in motivation; these constitute consummatory behaviors such as copulation. Other more variable behaviors allow an individual to converge on this functional outcome; these are appetitive behaviors such as seeking out a female (Timberlake and Silva, 1995). Experimental studies have revealed dissociations between aspects of the neural system regulating appetitive and consummatory components of male sexual behavior. Japanese quail (*Coturnix japonica*) readily exhibit sexual behavior and related social behaviors in captive conditions and have therefore proven valuable for studies of how early social experience can shape adult mate preference and sexual behavior. In addition they have also proven to be a good guide for researching how differences in photoperiod and photo colors control reproduction (Ball and Balthazart 2010). Four weeks after rearing under different lighting colors, three males from each group tested three times for the presence of male-typical copulatory behavior using behavioral procedures that have been modified from (Balthazart and Schumacher, 1984). Briefly, male from certain light color treatment was introduced into a small arena (50 × 60 cm) that contained a unfamiliar sexually mature female from different light treatment (10 weeks of age), and Copulatory activities happened within 15 minutes of the experiment were recorded [neck grabs, mounts, and cloacal contact movements (Adkins and Adler, 1972). Light -treated male that failed to exhibit mounts and cloacal contact movements were recorded as negative result. The subjects that exhibited high levels of copulatory behavior, indicating the best photosexual stimulatory effect of this light color than the other light colors and recorded as positive result. Even effect of different light colors on sexual behavior of female quails not studied.

2.4. Tonic immobility (TI) test

Tonic immobility duration was measured for six birds per each light treatment. The birds were taken to a separate room and were subjected to TI measurements. The TI was induced by inverting the bird on its back and applying a manual restraint until the bird stopped struggling. The TI duration was defined as the length of time during which the bird remained immobile (length of time from the moment the bird became immobile until the bird righted itself) after it had been held on its back for 10 s to induce the immobility reaction (Hoekstra *et al*. 1998). Unnecessary noise or movement was avoided. Direct eye contact between the observer and the bird was also avoided as it has been shown to prolong TI duration (Jones1986).The measure of TI was terminated if the bird had remained immobile for 300 s (TI = 300) or if it failed to remain at all immobile after five attempts of inducing TI (TI = 0). The number of attempts required to perform TI was recorded for each individual quail. In the TI test, the innate behavior provoked by the physical restraint of the bird corresponds to a catatonic-like state that is supposed to decrease the attention of the predator (Jones and Faure 1981).The lower the number of the attempts that are required reduced catatonic-like state the longer its duration the higher level of fear (Gallup 1979).

2.5. Statistical analysis

Data were tested for distribution normality and homogeneity of variance. Data were reported as means and pooled standard deviation. Data were analysed by one-way ANOVA with statistical package Minitab® software version 17. The significance of difference among the different light groups was evaluated by Tukey's test. In case of social proximity response, a Chi-square test was used. The significance level was set at $P \leq 0.05$.

RESULTS

Behavior of quails.

Table 1 showed the effect of different monochromatic lighting on the behavior of quails. The results showed that, except for drinking activity and sexual display in blue light group, there was no major ($P > 0.05$) disparity between light treatments on quail behavior.

3.2. Tonic immobility test.

The effect of different monochromatic lights on tonic immobility test is presented in table 2 and figure 1. The results showed that there was no significant ($P > 0.05$) difference between light treatments on the tonic immobility induction. While, there was a significant ($P \leq 0.05$) difference between light treatments on the tonic immobility duration. The longest tonic immobility duration was recorded in white light followed by green light, yellow light and red light. However the shortest tonic immobility duration was recorded in quails reared under BL color.

3.3. Social proximity response.

Table 3 showed the effect of various monochromatic lights on the response of social proximity, where negative impact of blue, green and white color of light on consummatory component of male sexual behavior i.e. copulatory behavior, that was clear by overall view of numbers of unsuccessful mating of males reared under these light colors, while yellow light color gave intermediate sexual response, compared with red light treatment which, showed the highest number of successful matings which confirmed the strong relationship between red light color and sexual behavior, due to its long wavelength and easier penetration of hypothalamus of quails.

Table 1: **effects of light colors on behavior of quails**

	Light color					PSD	*P*-value
	WL	GL	YL	RL	BL		
Drinking	0.500[ab]	0.507[ab]	0.410[b]	0.438[b]	0.615[a]	0.768	0.016*
Feeding	2.753	2.587	2.490	2.472	2.569	1.841	0.375
Agonistic	0.073	0.076	0.059	0.118	0.083	0.303	0.193
Crouch	5.972	5.753	6.139	5.767	5.927	3.428	0.647
Huddling	1.899	2.212	2.059	1.920	2.021	2.629	0.624
Standing	2.142	2.007	2.010	1.958	1.868	1.606	0.354
Walking	2.295	2.094	2.222	2.257	2.201	1.526	0.582
Running	0.135	0.097	0.087	0.125	0.139	0.491	0.625
St. wings	0.236	0.222	0.264	0.222	0.243	0.480	0.827
Wing &leg	0.260	0.236	0.215	0.2014	0.174	0.452	0.188
St. legs	0.219	0.198	0.146	0.122	0.177	0.438	0.057
Ruffling	0.108	0.142	0.115	0.132	0.142	0.356	0.677
Shaking	0.247	0.212	0.201	0.222	0.240	0.478	0.776
Preening	1.191	1.170	1.306	1.184	1.215	1.041	0.539
Pecking (all types)	0.399	0.427	0.358	0.375	0.392	0.643	0.753
Sexual activity	0.045[b]	0.098[a]	0.059[b]	0.049[b]	0.101[a]	0.029[c]	0.039*

*Asterisks indicate significant differences between experimental groups (one-way ANOVA *$p \leq 0.05$). Means within raw lack common superscripts differ significantly (Tukey's multiple comparison test, $P \leq 0.05$). PSD= pooled standard deviation

WL= white light; GL= green light; BL=blue light; RL= red light; YL= yellow light.

Table 2: effects of light colors on tonic immobility test of quails

	Light color					PSD	*P*-value
	WL	GL	YL	RL	BL		
TI induction	1.000	2.000	1.833	1.000	1.000	0.658	0.200
TI duration (s)	110.71[a]	65.76[b]	37.90[c]	20.21[d]	16.45[d]	7.340	0.000

Means within raw lack common superscripts differ significantly (Tukey's multiple comparison test, $P \leq 0.05$). PSD= pooled standard deviation

TI= tonic immobility; WL= white light; GL= green light; BL=blue light; RL= red light; YL= yellow light.

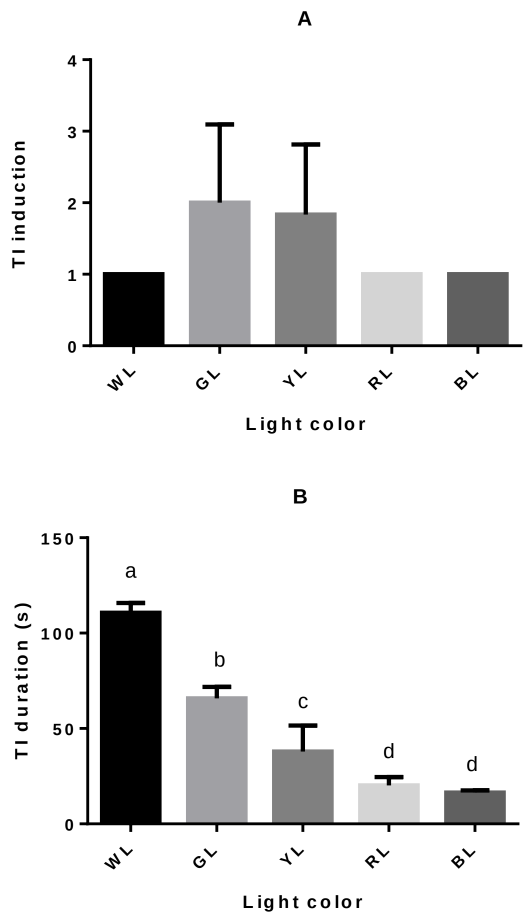

Figure 1: effects of light colors on tonic immobility test of quails (mean ± SD); A, TI induction numbers, B, TI duration (s).

Means lack common superscripts differ significantly (Tukey's multiple comparison test, $P \leq 0.05$).
TI= tonic immobility; WL= white light; GL= green light; BL=blue light; RL= red light; YL= yellow light.

Table 3: effects of light colors on social proximity response of quails

Cross mating	Mating behavior		Total	*P*-value
	Successful	Unsuccessful		
BM×GF	0	6	6	0.055
BM×RF	0	6	6	0.055
BM×WF	1	5	6	0.055
BM×YF	0	6	6	0.024*
GM×BF	1	5	6	0.024*
GM×RF	0	6	6	0.024*
GM×WF	0	6	6	0.055
GM×YF	0	6	6	0.055
RM×BF	3	3	6	0.873
RM×GF	1	5	6	0.873
RM×WF	0	6	6	0.024*
RM×YF	3	3	6	0.055
WM×BF	0	6	6	0.055
WM×GF	0	6	6	0.055
WM×RF	0	6	6	0.024*
WM×YF	1	5	6	0.055
YM×BF	1	5	6	0.055
YM×GF	0	6	6	0.024*
YM×RF	1	5	6	0.055
YM×WF	0	6	6	0.055
Total	12	108	120	1.000

W= white light; G= green light; B=blue light; R= red light; Y= yellow light; M= male; F= female
*Asterisks indicate significant differences between experimental groups (chi-square test *$p \leq 0.05$).

DISCUSSION

The findings of this study support the idea of stimulatory effect of light color with variable effects on quail behavior. Data presented in table (1) revealed that rearing Japanese quail under white light on one hand, it increased percentage of feeding, standing, movement activities, wing and leg stretch and shaking behaviors, moreover, red light increased agonistic behavior of quail. Similarly, Prayitno et al., (1997) suggested that birds reared in red or white light were more active, as expressed by greater walking activity in the white light treatment and by greater aggression in the treatment of red light. In contrast, Senaratna *et al.,* (2011) stated that light colors had no effect on eating behavior. Moreover, abnormal behavior (aggression and feather pecking) were significantly higher in chickens raised in red light color than in other colors due to increased behavior in poultry under red light shade, this may be attributed to light exposure and may affect the ability to deal with stressors (Campo et al., 2007). On other hand, the longest tonic immobility duration was recorded in white light because, the light color have an effect on brain organization that impact the behavioral responses, including fear behavior (Dharmaretnam and Rogers, 2005). This result showed the same finding of Mohamed et al., (2015) who stated that birds of white light displayed longer durations of tonic immobility compared with those of blue treatment.

Prayitno et al., (1997) suggested that the pineal gland's perception of long wavelength light is central to the activity effect. In addition to Hartwig and van Veen, (1979) who reported that in comparison to short wavelengths, long wavelengths entered the avian skull and induced reproductive production. This rise in penetration might clarify the results on performance, because increased mating behavior was an essential part of reproductive growth in many animals. These result similar to this study, which revealed that quails reared under red light showed significant positive mating results with unfamiliar females from other light treatments followed by yellow and white light colors. According to Blatchford et al., (2012) the absorption of red wavelength radiation in the hypothalamus is more sexually stimulating than green or blue wavelength. This could justify the better performance of the birds under the red LED, white LED and incandescent lamp, as they had red in their visible spectrum. Incandescent light has a red light aspect, whereas white fluorescent light has a bluish aspect, according to Mendes et al., (2010). The reason for this was that incandescent light produced longer wavelengths, near red, while fluorescent light produced shorter wavelengths, closer to green and blue. White, though, was a homogeneous combination of all shades. While crouching, wing stretch and preening behaviors tended to be higher in quails receiving yellow light. Similar result showed by Karousa, (1996) who stated that birds reared under red light had significantly higher percent of resting behavior, this attributed to rhymthic activity which was a feature of endocrine system. On one hand, our results disagreed with Praytino et al., (1997) and Mahrous et al., (1999) in point that birds reared under red light spent more time in wing stretching and preening, moreover, they agree with the same authors in point that stretch wings and preening behaviors showed lowest percentage in case of green light color. On other hand, the significant difference for different light colors on wing/leg stretch, leg stretch, ruffling and shaking were not noticed in the present study.

Green light group had higher exploratory, huddling behaviors, as that observed by Huber-Eicher, (2013) birds under green light spent much more time in pecking objects than birds under white light ,while Prayitno *et al.,* (1994) published the cage pecking was decreased in green light. Clearly, the intensity of a light source as well as illuminance may affect elements of explorative conduct. In addition, huddling behavior could be attributed to the calming effect of the light color of the short wavelength.

Ruffling behavior, sexual display and short tonic immobility duration were recorded in quails reared under blue, so this study supported the theory that blue color created a calming effect on birds, where the frequencies of physical activities was lower in chambers lighted with blue light, that may be attributed to the fact that blue light color alleviated the stress response in broilers through the reduction in the level of serum interleukin-1, as described by Xie et al., (2008) and Mohamed et al., (2014).

CONCLUSION

It was concluded that, rearing Japanese quails (*Coturnix coturnix japonica*) under blue light is preferable due to its soothing effect on bird's more than the other light colors, although red light has the best photosexual stimulatory effect compared to the other light colors.

CONFLICT OF INTEREST

The authors declared that present study was performed in absence of any conflict of interest.

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AUTHOR CONTRIBUTIONS

All authors contribute equally in this study.

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