

Targeted differential illumination improves reproductive traits of broiler breeder males

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ABSTRACT Artificial targeted illumination has a pivotal role in reproductive processes of poultry. The light-absorption mechanism in birds consists of 2 main components: the eye (retinal photoreceptors) and extraretinal photoreceptors located in the brain. Previous studies conducted on hens have shown that photostimulation of brain extraretinal photoreceptors elevates reproductive activity, whereas retinal photostimulation suppresses it. We tested the effect of targeted differential photostimulation (**TDP**) on reproductive activities of broiler breeder males. Fifty broiler breeder roosters (Ross), 21 wk of age, were divided into 5 environmentally controlled light-treatment rooms (n = 10) equipped with individual cages. Rooms 1 and 2 had 2 parallel lighting systems consisting of red light (630 nm) and green light (514 nm), and rooms 3 and 4 had parallel red and blue (456 nm) lighting systems. Room 5, illuminated with white light, served as the control. Birds of all groups were kept under short day (6L:18D) for 2 wk with both lighting systems in each treatment room turned on. At

23 wk of age, birds were photostimulated by gradually increasing one of the lighting systems to 14 h of light in each room, while the other lighting system was left on short day (6L:18D). Weekly semen samples were collected until 65 wk of age and analyzed for volume, motility, concentration and vitality. Monthly blood samples were drawn for plasma hormone assays. At 65 wk of age, roosters were euthanized and hypothalamus, pituitary gland, retina and testes samples were taken for mRNA expression analysis. TDP using long-day red light and short-day green light significantly increased reproductive performance, manifested by higher semen volume, motility and concentration, and testis weight; furthermore, this group had higher plasma testosterone levels, higher GnRH mRNA expression in the hypothalamus, lower levels of aromatase in the testes, and lower mRNA expression of hypothalamic serotonin transporter, and of pituitary prolactin and its receptors in the testes. This is the first study showing a positive effect of TDP on reproduction of broiler breeder roosters.

Key words: photostimulation, extraretinal photoreceptors, broiler breeder males, semen, reproduction

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INTRODUCTION

The last few decades have seen enormous investment in genetic modification of broiler breeders for increased breast muscle, to meet the growing demand for poultry products worldwide (Chai et al., 2017). From 1957 to 2007, broiler growth rate increased by 400% (Zuidhof et al., 2014).

The increased weight and size of broiler breeders has had a negative impact on the reproductive aspects of

heavy breeding (Decuyper et al., 2010). Studies have shown a negative correlation between high body weight and reproductive performance, including poor semen quality in males (Reddy and Sadjadi, 1990) and an increase in erratic ovulation and defective eggs in females (Udale et al., 1972; Hocking et al. 1987, 1989). Thus, ways to increase reproduction in broiler breeders that does not interfere with growth rate are being sought. One solution may lie in the use of targeted differential photostimulation (**TDP**) as a reproduction accelerator.

The photoabsorption mechanism in birds consists of 2 major sites: the retina in the eye and various locations in the brain (Menaker and Underwood, 1976), including the hypothalamus (Scanes, 2014). The retinal photoreceptors consist of rods, which assist in vision under poor lighting conditions, and cones which are more efficient in bright light and allow absorption and perception of

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color (Philips Lighting, 1988). The different types of retinal cones cover the violet (or ultraviolet), blue, green and red sections of the visible spectrum (Pritchard, 1995). Photons of light absorbed through these photoreceptors are converted into neural signals through photochemical changes in the retina whereby opsin-protein complexes are isomerized after binding with vitamin A (Hart, 2001). These signals are used to activate, enhance or decelerate many biological processes in chickens (Applebury and Hargrave, 1986). However, studies have shown that in domestic fowl, among other bird species, in contrast to mammals, longer wavelengths are capable of penetrating directly through the skull and tissues to the hypothalamus, which contains photoreceptors known as extraretinal photoreceptors (ERPR) (Foster and Hankins, 2002).

In fowl, photostimulation caused by exposure to longer periods of light is a key factor in inducing reproduction and increasing its rate (Mobarkey et al., 2010). This is achieved through control of gonadotropin-releasing hormone (GnRH) secretion, which triggers cascading endocrine stimulation of the gonadal axis. In the gonadotropic axis, the hypothalamic GnRH activates the pituitary gland to release luteinizing hormone (LH) and follicle-stimulating hormone (FSH). These gonadotropins increase the production and release of gonadal hormones (Benoit, 1978).

There is some evidence for a correlation between exposure of the ERPR to longer wavelengths of the light spectrum (red illumination) and stimulation of the reproductive axis (Lewis and Morris, 2000), whereas the photoreceptors in the retina, which mostly absorb the shorter wavelengths of the spectrum (blue-green light), stimulate the release of gonadotropin inhibitory hormone, thereby decreasing reproduction (Benoit and Assenmacher, 1966; Mobarkey et al., 2010). Previous studies conducted in our laboratory combined the 2 factors that can manipulate reproduction in poultry: duration of illumination and its wavelength. Photostimulation was used to activate the ERPR, while maintaining the retinal photoreceptors under nonphotostimulatory conditions. This system enhanced reproduction in broiler breeders and turkey hens (Mobarkey et al., 2010; Rozenboim and El Halawani, unpublished data).

The effects of wavelength and exposure duration on male reproductive traits of domestic fowl have been much less studied than in females. Stimulation of sexual maturation in males has been shown to be greatest under illumination with white light or the red part of the spectrum in fowls (Johnson et al., 1982), turkeys (Benoit et al., 1950), and quails (Woodard et al., 1969). In roosters, inhibited spermatogenesis and GnRH levels, and lower testis weight were observed under illumination with red or white light for 6 h as compared to 14 h. Illumination in the same manner with green or blue light did not have the same effect (Casey et al., 1969). Although the quality of male semen—sperm concentration, viability, and motility—plays a key role in reproduction, and a problem with any one of these 3

parameters can result in a subfertile rooster (McDaniel et al., 1998), there has been minimal research on the effect of differential photostimulation on reproductive traits of broiler breeder males.

The aim of this study was therefore to determine the effects of differential photostimulation on the reproductive system of broiler breeder males by applying ERPR photostimulation by red-light illumination (14 h), while maintaining nonphotostimulatory conditions for the retinal photoreceptors (illumination for 6 h with either blue or green light), and vice versa, and examining reproductive traits.

MATERIALS AND METHODS

Animals

All procedures were approved by the Animal Care and Welfare Committee of The Hebrew University of Jerusalem, Israel on 13 September 2016. Research number AG-16-14904-1. Fifty broiler breeder males (Ross 308) at 21 wk of age were divided into 5 equal-weight groups ($n = 10$, 3.39 ± 0.2 kg) and housed in 5 environmentally and light-controlled rooms equipped with individual rooster cages. Roosters were reared according to the Ross Parent Stock Management guide until the end of the experiment. Administration of feed was calculated according to age in weeks and BW of the roosters. Water was provided *ad libitum*. Rooms were light-proof, and all light was provided by LED lamps according to the light treatment.

Light Treatments

The control group was illuminated with a full light spectrum using warm white LED lamps (intensity of 0.1 W/m^2 ; 20 lux). The units of watts per square meter are a more suitable measure of light intensity when examining the system that provides skull-penetrating light (white and red). Lux units, on the other hand, measure brightness, and are therefore used for the eye-stimulating system (green and blue light). Two rooms had 2 parallel lighting systems: red light (wavelength of 630 nm, intensity of 0.1 W/m^2) and green light (514 nm, 20 lux). Two other rooms also had 2 parallel lighting systems: red light (630 nm, 0.1 W/m^2) and blue light (456 nm, 20 lux). The light spectrum was measured with UPRtek MK350S Handheld Spectrometer (MK350S Handheld Spectrometer, UPRtek, Taiwan) and light intensities were determined by LI-COR light meter (Lincoln, NE).

For the first 2 wk of the experiment, the roosters were kept under nonphotostimulatory conditions (6L:18D) with all illumination systems on simultaneously. At the age of 24 wk, light hours were gradually increased and changed in all rooms to achieve photostimulatory conditions: in the control room, light hours were added until long-day (LD) conditions (14L:8D) were achieved. In the other 4 treatment rooms, light hours of one of the lighting systems were increased to long day (LD, 14L), whilst the other lighting system in the room remained

Table 1. Lighting regime and treatments.

Treatment	White light	Blue light	Red light	Green light	Total h
Control group	0700 – 2100 h	-----	-----	-----	14
Blue-Red group	-----	0700 – 2100 h	0700 – 1300 h	-----	14
Red-Blue group	-----	0700 – 1300 h	0700 – 2100 h	-----	14
Green-Red group	-----	-----	0700 – 1300 h	0700 – 2100 h	14
Red-Green group	-----	-----	0700 – 2100 h	0700 – 1300 h	14

on short day (SD, 8L). [Table 1](#) provides the details of the lighting regimes.

The lighting treatments were divided into LD and SD to create a differential stimulatory or nonstimulatory effect on the retinal and extraretinal photoreceptors. As retinal photoreceptor sensitivity is highest in the green and blue part of the spectrum ([Mobarkey et al., 2010](#)), we used SD green and blue lighting with LD red lighting to reduce retinal stimulation. Conversely, SD red lighting was used along with LD blue/green lighting to reduce or completely prevent photostimulation of the ERPR.

Semen Quality

Individual semen quality analysis was conducted weekly from 31 to 65 wk of age. Semen volume was measured in scaled (mL) test tubes. Motility was evaluated by scoring the motility rate from 1 (no motility) to 8 (fluent motion with turbulence), when the rating between is -very poor motility rate of cells in place (2), medium motility of the cells in place (3), cells with vigorous tail movement without motion forward (4), medium motility with motion forward (5), vigorous motion of the cells with motion forward (6) and fluent motion without turbulence (7). Sperm cells were counted in a hemocytometer by adding 20 μ L semen to 5 mL distilled water, and the final concentration of sperm cells per milliliter was calculated as: number of cells counted \times 201 \times 100,000. Concentration per ejaculate was calculated as: concentration per milliliter \times volume of the rooster's ejaculate. Viability was analyzed by Eosin-Nigrosin staining of 10 μ L semen immediately after

ejaculation; the number of dead cells was counter, and percent mortality was calculated manually using a microscope.

Blood Sampling

Heparinized blood samples were taken from all roosters once every 4 wk and the plasma was stored at -20°C until assay. All blood drawings were performed at the same time at 9 am pre-feeding. The plasma was analyzed for prolactin, estradiol, progesterone and testosterone levels by competitive enzyme linked immunosorbant assay (**ELISA**).

Hormone Analysis

For the competitive ELISA of plasma prolactin levels, plates (MaxiSorp, Nunc Immuno MicroWell, Sigma-Aldrich, Rehovot, Israel) were coated with goat anti-rabbit IgG, 0.1 mL per well (Jackson ImmunoResearch Laboratories, West Grove, PA). The coating was diluted 1:2,000 in 0.05 M potassium phosphate buffer pH 7.4 and incubated overnight at 4°C. Then the plates were washed 3 times in 0.03 M Phosphate Buffered Saline (**PBS**) containing 0.05% Tween 20. To dilute the samples, we used an assay buffer containing 0.15 M PBS pH 7.2 with 0.1% casein, 1 mM EDTA and 0.02% thimerosal. A 40- μ L aliquot of assay PBS was added to each 10 μ L of sample or standard containing 0.039 to 20 ng prolactin (A. F. Parlow, National Hormone and Peptide Program, Harbor-UCLA R.E.I., Torrance, CA) and the mixture was pipetted into the wells. A 25- μ L aliquot of

Table 2. Primers used in real-time PCR.

Gene	Primers (5'→3')	Product length	GenBank accession no.
GAPDH	R:CCTGCATCTGCCCATTT F:GGCAGCCATCACTATC	61	NM_204,305.1
Actin	R:AAAGCCATGCCAATCTCGTC F:CCGCAAATGCTTCTAAACCG	101	NM_205,518.1
Aromatase	R:AGCTGTTAGGCAACTGTGGA F:CCAGTTGCCACAGTGCCTAT	114	
GnRH-1	R:GATCAGGCTTGCCATGGTTT F:TGGTCTTATGGCCTGCAACC	174	NM_001080877.1
LH	R:CCCATAAGTGACGCCG F:GAATGCCCCCAATGTATGGCT	118	S_70834
FSH	R:ATTCAGGATGGTCACCCGAG F:CTGCTTCACAAGGGATCCAGTA	122	NM_204257.1
LH receptor	R:GGTAGGTCAGAACGGCTTCC F:GGCCAGACGTCTGGATATT	159	NM_204936.1
FSH receptor	R:GGACAAATCTCAGTTCTGTGGC F:GGACAAATCTCAGTTCTGTGGC	154	NM_205079.1
Serotonin transporter	R:CGCTTGCTCCAGACGTGT F:CCGAAGCCATTGCGAACA		NM_213572.1

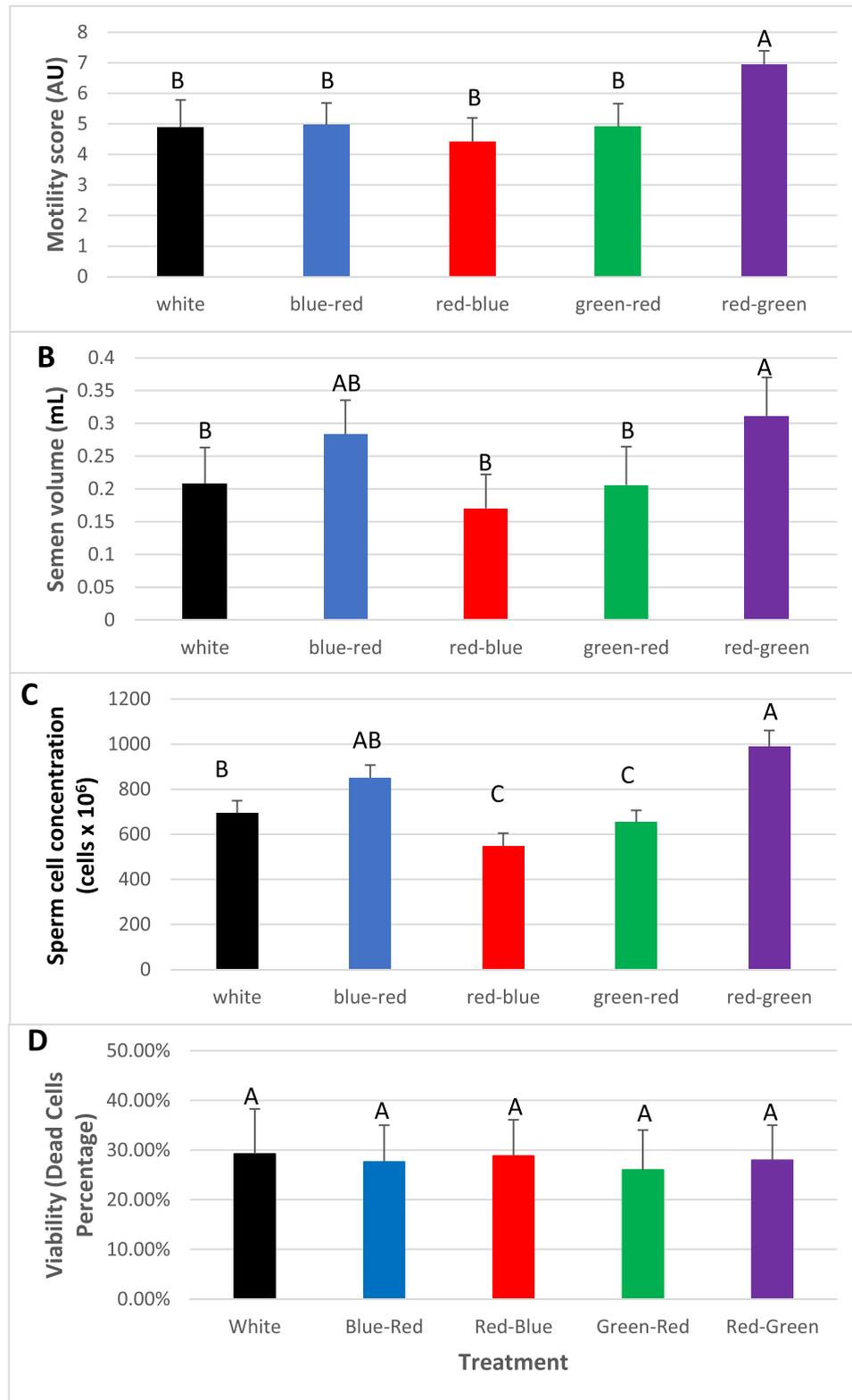


Figure 1. Cumulative semen analysis for total duration of the experiment. Broiler breeder roosters exposed to: 14 h white light, control (white); blue light for 14 h combined with 6 h red light (blue-red); red light for 14 h with 6 h blue light (red-blue); green light for 14 h with 6 h red light (green-red); red light for 14 h with 6 h green light (red-green). (A) Motility rate in all groups. (B) Semen volume. (C) Concentration of sperm cells per ejaculate. Data are presented as average \pm standard error. Levels with different letters are significantly different ($P \leq 0.05$). (D) Viability of sperm cells. Data are presented as average \pm standard error. Levels with different letters are significantly different ($P \leq 0.05$).

biotinylated prolactin diluted 1:250,000 was added to each well along with 25 μ L rabbit anti-chicken prolactin diluted 1:250,000 (for broader phylogenetic recognition of avian prolactin). The antibody was validated for

poultry, with parallel curves starting at 5 μ L achieved for chicken (Israel Rozenboim, unpublished data). The plates were left overnight at 4°C, then washed and 0.1 mL of streptavidin–horseradish peroxidase (1:5,000)

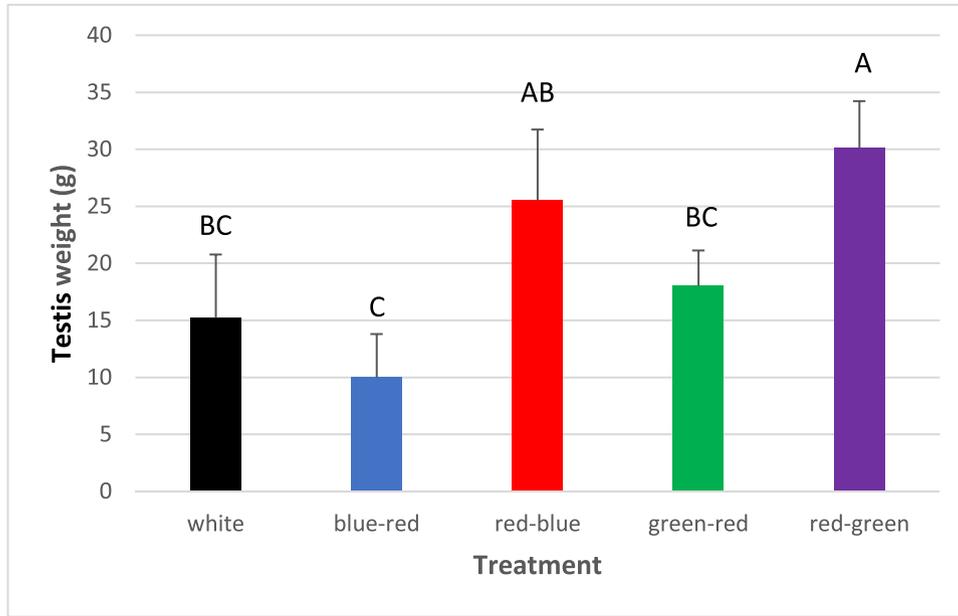


Figure 2. Weight of testes of all roosters after exposure to: 14 h white light, control (white); blue light for 14 h combined with 6 h red light (blue-red); red light for 14 h with 6 h blue light (red-blue); green light for 14 h with 6 h red light (green-red); red light for 14 h with 6 h green light (red-green). Data are presented as average \pm standard error. Levels with different letters are significantly different ($P \leq 0.05$).

was added. The plates were incubated for 2 h at room temperature and then washed, and 0.1 mL ABTS reagent (0.04% 2,2'-azino-bis-3-ethylbenzthiazoline-6-sulfonic acid and 0.015% H_2O_2 in 0.1 M citrate phosphate buffer at pH 4) was added. After 30 min of incubation at room temperature, the reaction color was measured at 405 nm in a Tecan Sunrise ELISA reader (Tecan Group Ltd., Männedorf, Switzerland). The measurement was conducted in duplicates; intra-assay CV with pooled poultry plasma was 7% and inter-assay CV was not computed as all samples were analyzed in a single assay.

Steroid analysis (progesterone, estradiol and total testosterone) was conducted as previously described

(Nash et al., 2000), using primary antibody and tracer dilutions, respectively, of 1:5,000 and 1:50 for progesterone, 1:160,000 and 1:160 for estradiol, and 1:320,000 and 1:320 for testosterone. All samples were analyzed in duplicate, and a separate standard curve was determined for every other plate.

Tissue Sampling

At the end of the experiment, all roosters were euthanized. The brain was extracted from the skull and the entire hypothalamus was sampled, followed by the

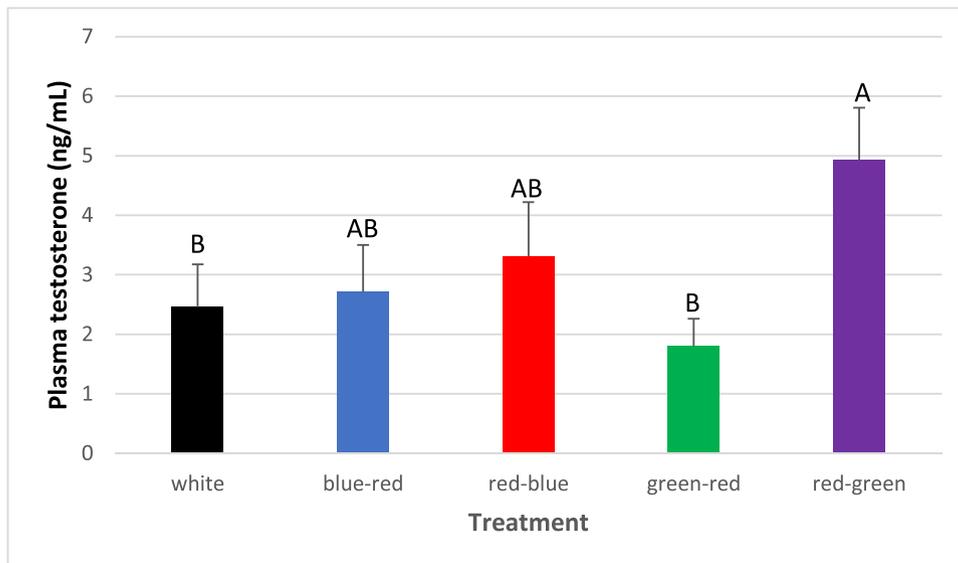


Figure 3. Cumulative plasma testosterone levels for the total duration of the experiment. Broiler breeder roosters exposed to: 14 h white light, control (white); blue light for 14 h combined with 6 h red light (blue-red); red light for 14 h with 6 h blue light (red-blue); green light for 14 h with 6 h red light (green-red); red light for 14 h with 6 h green light (red-green). Data are presented as average \pm standard error. Levels with different letters are significantly different ($P \leq 0.05$).

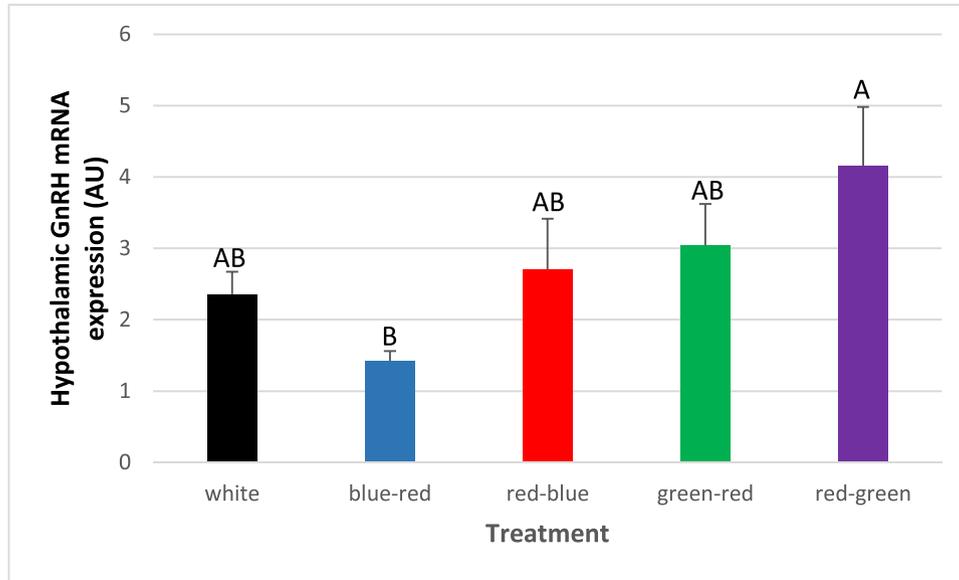


Figure 4. Hypothalamic GnRH mRNA levels in broiler breeder roosters exposed to: 14 h white light, control (white); blue light for 14 h combined with 6 h red light (blue-red); red light for 14 h with 6 h blue light (red-blue); green light for 14 h with 6 h red light (green-red); red light for 14 h with 6 h green light (red-green). Data are presented as average \pm standard error. Levels with different letters are significantly different ($P \leq 0.05$).

pituitary gland. The retina was collected from one of the rooster's eyes. Testes were weighed and a tissue sample was collected as well. All collected tissues from all groups were removed simultaneously, placed in liquid nitrogen and stored at -80°C until gene-expression analysis at the mRNA level.

RNA Extraction and Real-Time PCR

The procedure was conducted as described in Dishon et al. (2017). Briefly, frozen samples were homogenized, and total RNA was extracted using RNazol RT reagent (Genecopoeia, Rockville, MD). Concentration was measured by NanoDrop ND-1000 UV-Vis

spectrophotometer (NanoDrop Technologies, Wilmington, DE). A reverse transcription reaction was performed to produce a total $20 \mu\text{L}$ cDNA. Real-time PCR was conducted using β -actin and glyceraldehyde 3-phosphate dehydrogenase (GAPDH) as the standards. The sequences of all gene-specific primers used in the PCR are shown in Table 2. Gene-expression results are presented in arbitrary units (AU).

Statistical Analysis

To determine the necessity of a repeated measures test, a correlation test was performed on all routine analyses for semen quality and plasma steroids. No correlation was found between rooster age and treatment.

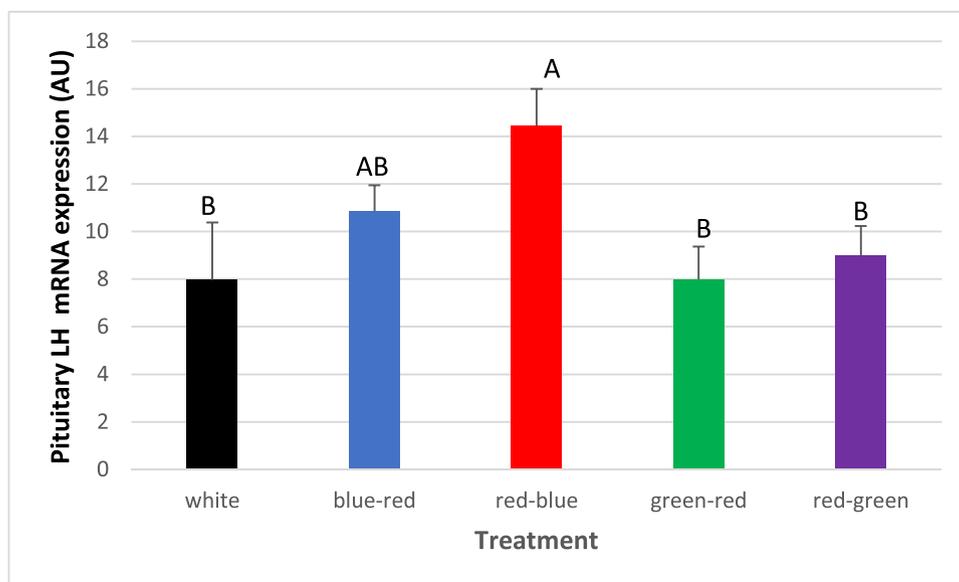


Figure 5. Pituitary LH mRNA levels in broiler breeder roosters exposed to: 14 h white light, control (white); blue light for 14 h combined with 6 h red light (blue-red); red light for 14 h with 6 h blue light (red-blue); green light for 14 h with 6 h red light (green-red); red light for 14 h with 6 h green light (red-green). Data are presented as average \pm standard error. Levels with different letters are significantly different ($P \leq 0.05$).

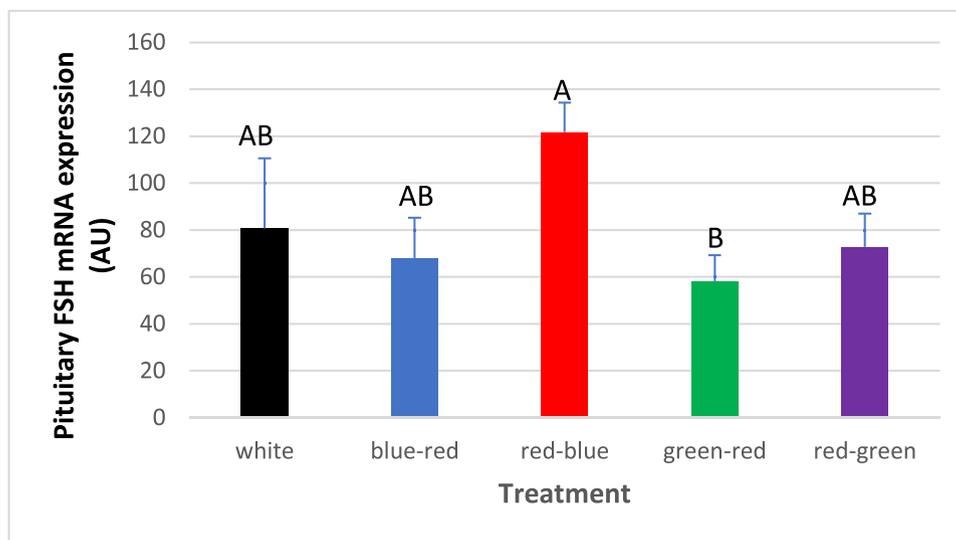


Figure 6. Pituitary FSH mRNA levels in broiler breeder roosters exposed to: 14 h white light, control (white); blue light for 14 h combined with 6 h red light (blue-red); red light for 14 h with 6 h blue light (red-blue); green light for 14 h with 6 h red light (green-red); red light for 14 h with 6 h green light (red-green). Data are presented as average \pm standard error. Levels with different letters are significantly different ($P \leq 0.05$).

All data were analyzed by one-way ANOVA with treatment (Lighting) as fixed effect. We used Tukey-Kramer HSD test as a post-hoc testing of the differences between means least square means. The differences were considered significant at $P \leq 0.05$. Data were analyzed using JMP14 software (SAS Institute, Cary, NC, 2000)

RESULTS

Semen Analysis

Weekly semen quality results were averaged for the whole experimental period to determine the overall impact of TDP treatments on semen quality of broiler breeder roosters. Significant elevation in all 3 quality parameters was observed in the Red-Green group compared to the control (Figure 1). Motility levels were

significantly higher in the Red-Green group than in all other groups, with an average score of 7.013 out of 8 compared to 4.9 for the other groups; ejaculate volume and concentration were slightly higher for the Blue-Red treatment group (966.1×10^5 sperm cells) compared to the control (727.11×10^5 sperm cells) and the other treatment groups except the Red-Green group, the latter being highest with an average $1,008.91 \times 10^5$ sperm cells per ejaculate.

Sperm cell viability was not significantly different between groups, and there was no positive or negative impact of the treatments on this parameter.

Testis Weight

The 2 LD red treatment groups (Red-Green and Red-Blue) had a positive impact on testis weight at the end

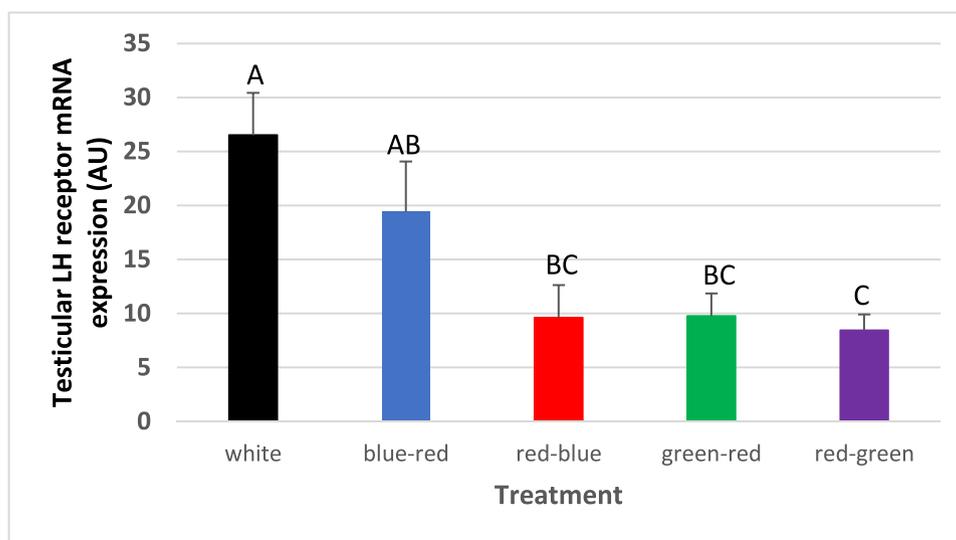


Figure 7. Testicular LH receptor mRNA levels in broiler breeder roosters exposed to: 14 h white light, control (white); blue light for 14 h combined with 6 h red light (blue-red); red light for 14 h with 6 h blue light (red-blue); green light for 14 h with 6 h red light (green-red); red light for 14 h with 6 h green light (red-green). Data are presented as average \pm standard error. Levels with different letters are significantly different ($P \leq 0.05$).

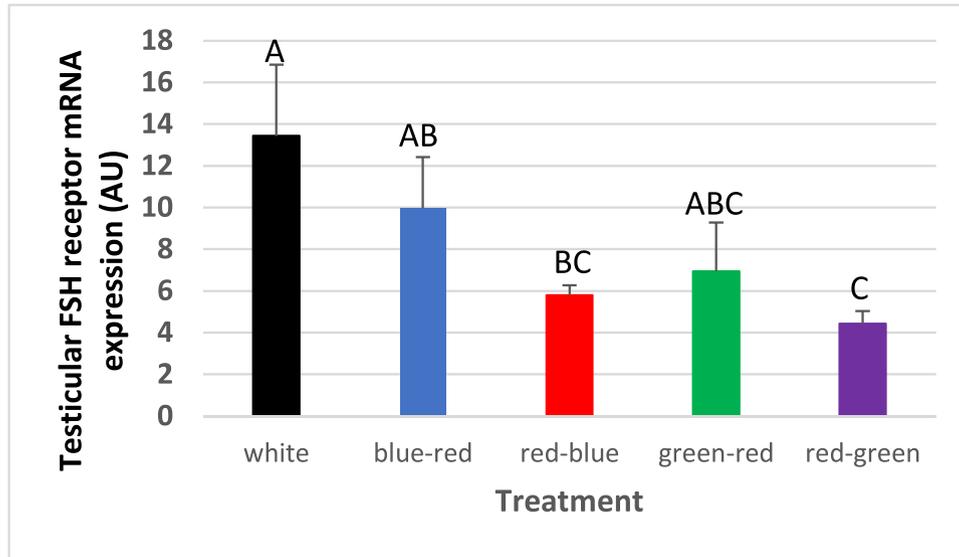


Figure 8. Testicular FSH receptor mRNA levels in broiler breeder roosters exposed to: 14 h white light, control (white); blue light for 14 h combined with 6 h red light (blue-red); red light for 14 h with 6 h blue light (red-blue); green light for 14 h with 6 h red light (green-red); red light for 14 h with 6 h green light (red-green). Data are presented as average \pm standard error. Levels with different letters are significantly different ($P \leq 0.05$).

of the experiment (65 wk of age). The highest testis weight was in the Red-Green group with an average of 30.1 g, and the Red-Blue group had a slightly lower average weight of 25.2 g. Both groups showed significantly higher weights than the LD green and LD blue groups and the control group, with an average testis weight of 15.2 g (Figure 2).

Plasma Steroid and Prolactin Levels

As with all of the semen analysis measurements, the monthly results of plasma steroid and prolactin levels were calculated to cumulative results, to show the impact throughout the entire experimental period, as no correlation between age and treatment was found. Although

plasma estradiol, prolactin and progesterone levels were not significantly different between the different treatments, the LD red light combined with SD green light resulted in elevated total plasma testosterone levels, to an average 4.99 ng/mL, compared to all other treatment and control groups that averaged 3.04 ng/mL (Figure 3). The difference was significant compared to the Green-Red treatment and control group.

Gonadotropic Axis Gene Expression

The Red-Green treatment resulted in a higher level of hypothalamic GnRH mRNA compared to all other treatment groups and the control group, significantly so compared to the Blue-Red treatment group (Figure 4).

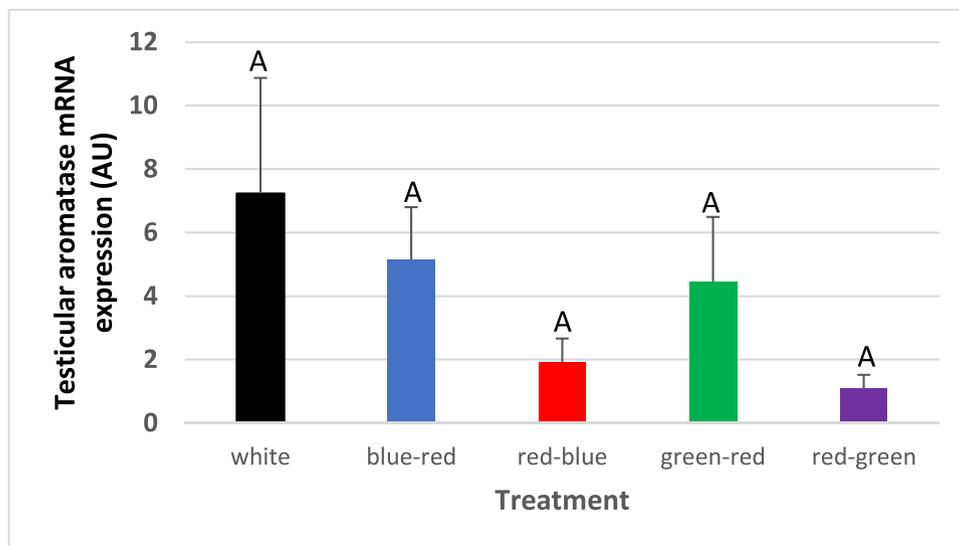


Figure 9. Testicular aromatase mRNA levels in broiler breeder roosters exposed to: 14 h white light, control (white); blue light for 14 h combined with 6 h red light (blue-red); red light for 14 h with 6 h blue light (red-blue); green light for 14 h with 6 h red light (green-red); red light for 14 h with 6 h green light (red-green). Data are presented as average \pm standard error. Levels with different letters are significantly different ($P \leq 0.05$).

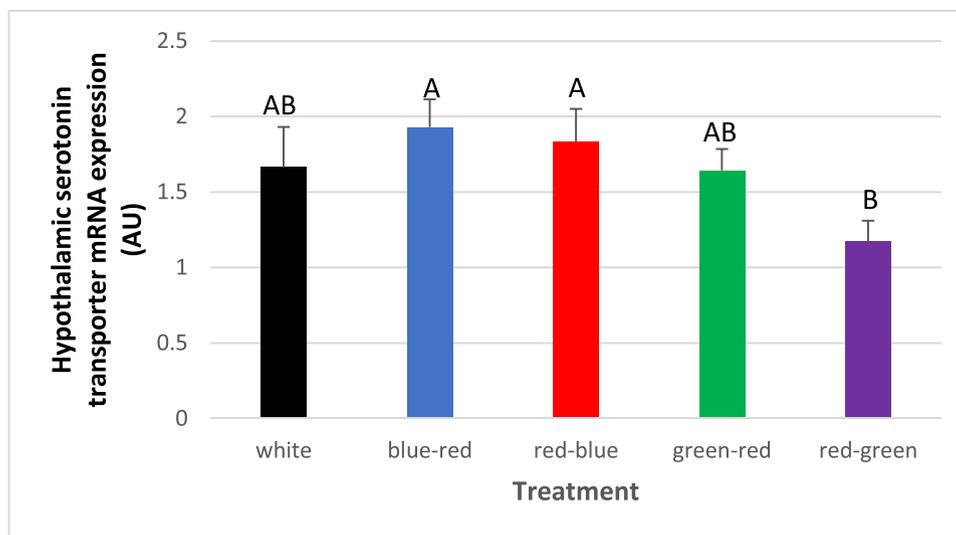


Figure 10. Hypothalamic serotonin transporter mRNA levels in broiler breeder roosters exposed to: 14 h white light, control (white); blue light for 14 h combined with 6 h red light (blue-red); red light for 14 h with 6 h blue light (red-blue); green light for 14 h with 6 h red light (green-red); red light for 14 h with 6 h green light (red-green). Data are presented as average \pm standard error. Levels with different letters are significantly different ($P \leq 0.05$).

LH mRNA level in the pituitary gland was higher in the Red-Blue group than in all other treatment groups and the control group, significantly so for all but the Blue-Red group (Figure 5). Levels of FSH mRNA in this tissue were highest in the Red-Blue group, but only significantly so compared to the Green-Red treatment group (Figure 6). Expression of LH and FSH receptor mRNA in the testes was lower in the Red-Green group than in all other treatment groups, and markedly so compared to the control group (Figures 7, 8). Aromatase mRNA levels in the testes were lowest in the Red-Green treatment group (Figure 9). Although the difference was not significant, the LD red treatments resulted in lower expression of this gene compared to all other treatments and most of all to the control, which showed the highest level of expression.

Serotonergic and Lactotrophic Axis mRNA Expression

Lower serotonin transporter mRNA expression in the hypothalamus was observed in the Red-Green treatment group. It was significantly lower than in the Blue-Red and Red-Blue treatment groups (Figure 10). Retinal serotonin transporter expression in this group was also lower than in all other treatment groups and the control (Figure 11). Furthermore, prolactin mRNA levels in the pituitary were significantly lower in the Red-Green group than in all other treatment groups and the control, except in comparison to the green-red group (Figure 12), along with lower prolactin receptor mRNA gene expression in the testes in the Red-Green and Red-Blue groups compared to the other treatment groups

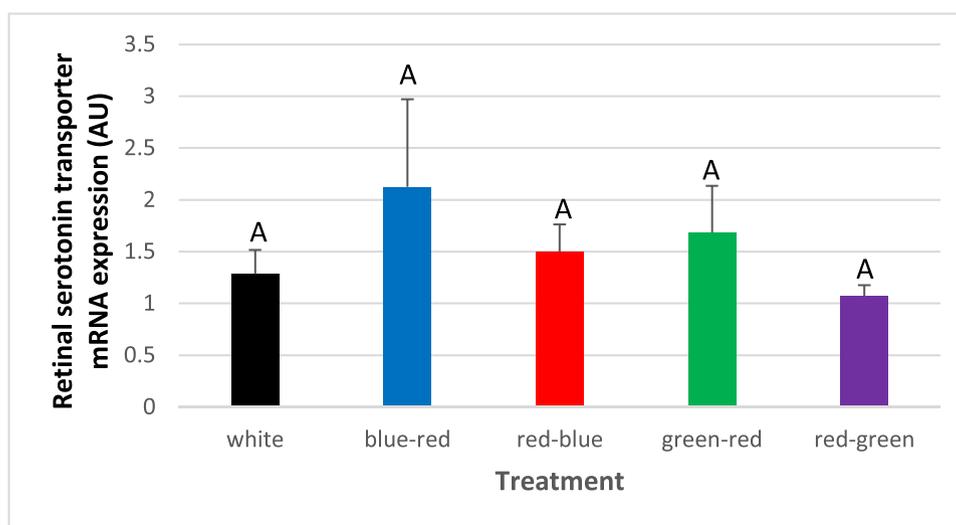


Figure 11. Retinal serotonin mRNA levels in broiler breeder roosters exposed to: 14 h white light, control (white); blue light for 14 h combined with 6 h red light (blue-red); red light for 14 h with 6 h blue light (red-blue); green light for 14 h with 6 h red light (green-red); red light for 14 h with 6 h green light (red-green). Data are presented as average \pm standard error. Levels with different letters are significantly different ($P \leq 0.05$).

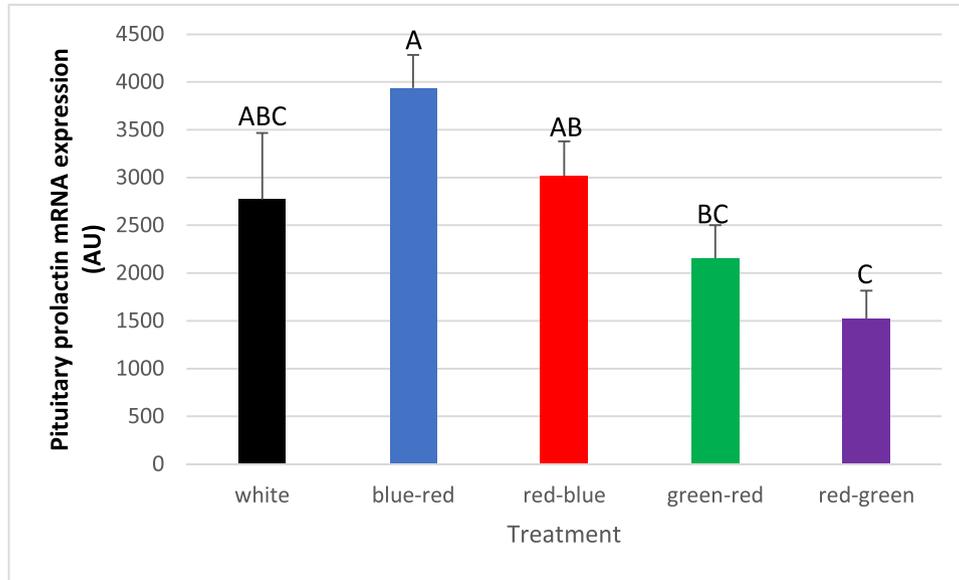


Figure 12. Pituitary prolactin mRNA levels in broiler breeder roosters exposed to: 14 h white light, control (white); blue light for 14 h combined with 6 h red light (blue-red); red light for 14 h with 6 h blue light (red-blue); green light for 14 h with 6 h red light (green-red); red light for 14 h with 6 h green light (red-green). Data are presented as average \pm standard error. Levels with different letters are significantly different ($P \leq 0.05$).

and significantly lower than control and the Blue-Red groups (Figure 13).

DISCUSSION

Reproductive traits of broiler breeder males were improved by TDP of the ERPR using red light illumination combined with nonphotostimulation of the retinal photoreceptors with green light illumination. The improvement was manifested by elevated levels of semen volume, motility, sperm cell concentration per

ejaculation, and testis weight. Furthermore, plasma testosterone levels were elevated in the Red-Green group compared to the other treatment groups and control, along with elevated expression of hypothalamic GnRH mRNA and lower mRNA expression of testicular aromatase and LH and FSH receptors. Moreover, expression of serotonin transporter mRNA in the hypothalamus and retina was lower in the Red-Green group than in all other treatment groups and the control, as was prolactin mRNA expression in the pituitary gland and that of its receptors in the testes.

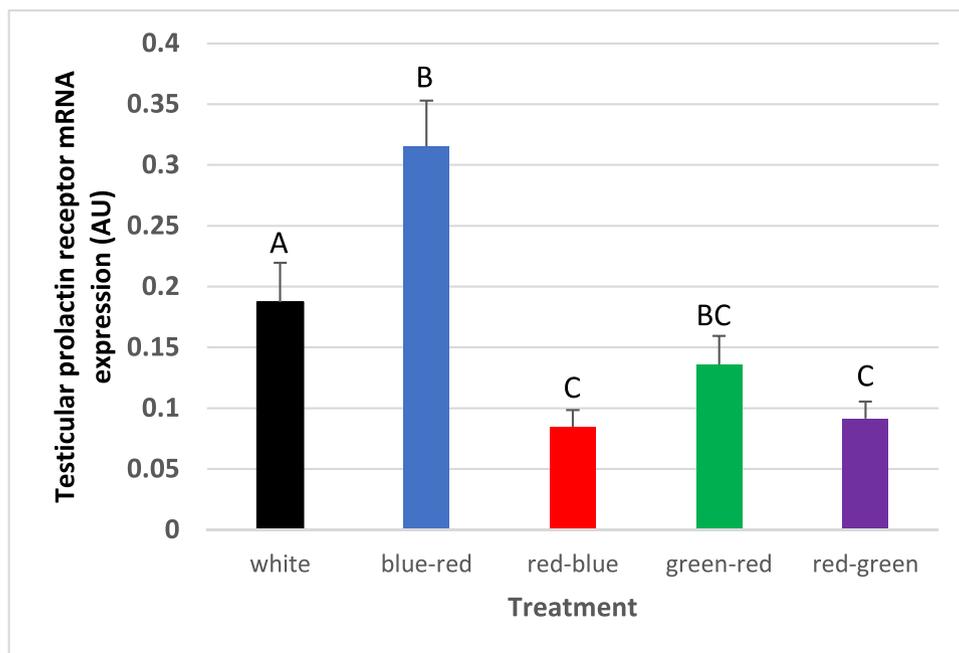


Figure 13. Testicular prolactin receptor mRNA levels in broiler breeder roosters exposed to: 14 h white light, control (white); blue light for 14 h combined with 6 h red light (blue-red); red light for 14 h with 6 h blue light (red-blue); green light for 14 h with 6 h red light (green-red); red light for 14 h with 6 h green light (red-green). Data are presented as average \pm standard error. Levels with different letters are significantly different ($P \leq 0.05$).

In recent years, the broiler breeder industry has increased the use of semen analyses as an indication of reproductive quality (Tabatabaei et al., 2009). Semen quality can be ranked using numerous characteristics which, together, indicate the quality of the tested sperm cells and seminal fluid. The most common attributes used are volume, motility, viability and concentration (McDaniel et al., 1998). There are very few studies published on the effect of monochromatic illumination with different wavelengths on the quality of semen in poultry. A previous study on male Japanese quails found a positive impact of red illumination on testis weight (Retes et al., 2017). In the present study, we not only found increased testis weight, but also significant elevation in most of the semen quality criteria tested in the Red-Green group.

Hypothalamic GnRH mRNA expression was elevated in the Red-Green treatment group compared to all other treatment groups and the control. Continuous exposure throughout the experiment to 14 h of red light resulted in continual GnRH formation and release. This correlates with previous studies conducted on female broiler breeders in which the longer wavelength illumination penetrated the skull and elevated the secretion of GnRH, triggering the hypothalamic–pituitary–gonadal axis (Zaguri et al., 2020; Saldanha et al., 2001; Mobarkey et al., 2009). In the gonadotropic axis, high levels of testosterone, as seen in this study, are expected to inhibit GnRH secretion and thus lower reproduction rate (King et al., 1989). However, even though LH and FSH receptor mRNA levels in the testes were at lower levels than in the control, hypothalamic GnRH mRNA remained at a higher level in the Red-Green treatment group. These results correlate with studies showing separate and direct inhibition by high levels of testosterone on LH and FSH levels, in contrast to the indirect inhibition through hypothalamic GnRH (Jenkins et al., 1978; Maung and Follett, 1978; Chase, 1982). Along with these results, aromatase mRNA level in the testes was the lowest in the Red-Green treatment group. Weil et al. (1999) stated that a low amount of aromatase in the testes results in low testicular estradiol, which increases reproduction in older roosters (Weil et al., 1999). Moreover, it has been shown that induced aromatase inhibitor can improve reproductive traits in aging roosters (Ali et al., 2017). Thus, low levels of aromatase might have contributed to the constant high levels of plasma testosterone in the Red-Green treatment group, resulting in higher testis weight and semen quality.

In addition to the gonadotropic axis that improved reproduction attributes of the Red-Green treatment group, levels of hypothalamic and retinal serotonin transporter mRNA were lower than for the other treatment groups and control. This result correlates with previous studies showing that high serotonin levels suppress reproductive-axis activity directly through suppression of GnRH synthesis and LH secretion in aging broiler breeder roosters (Avital-Cohen et al., 2015) and

turkeys (El Halawani et al., 1995), and indirectly via the lactotrophic axis in turkeys (Rozenboim et al., 1993).

In our study, mRNA expression levels of prolactin in the pituitary gland and its receptors in the testes were lower in the Red-Green group than in the other treatment groups and control. Avital-Cohen et al. (2013) found direct involvement of high prolactin levels in the pituitary with deterioration of reproductive traits in aging roosters. This was manifested by low semen quality, low plasma testosterone concentration and regression of the testes. As serotonin has a pivotal indirect role in prolactin production via the synthesis of vasoactive intestinal peptide, which is controlled by serotonin (El Halawani et al., 1988; Mobarkey et al., 2013), our results are, in fact, connected and may indicate a strong bond between the serotonergic, lactotrophic and gonadal axes that influences the improvement in reproductive attributes in broiler breeder roosters. Whereas in previous studies, serotonin and prolactin levels induced changes in gonadal axis hormones, in the current study, the origin of the shift was TDP which directly elevated GnRH secretion and thus triggered the entire axis and suppressed serotonin and prolactin levels. This indicates an inverse, but dual, relationship between the gonadal axis and the serotonergic and lactotrophic axes.

In conclusion, this is the first study to demonstrate an improvement in reproductive performance, including an increase in semen quality, as a direct outcome of targeted differential monochromatic illumination consisting of LD exposure to red light combined with SD exposure to green light. It shows the effect of TPD on 3 reproductive axes—gonadal, serotonergic and lactotrophic—and its contribution to improving reproductive attributes in broiler breeder males and enabling their conservation at an older age. Furthermore, this study demonstrates a lack of negative effects of the lighting treatment on reproductive aspects of broiler breeder males, especially considering the duration of the experiment—45 wk of rearing. However, further studies are required to elucidate the full mechanism of TDP's impact on the reproductive axes of the broiler breeder male, including reproduction and fertility per se. This study can be considered as a first step toward manipulating the effects of TDP on the male reproduction system in broiler breeding.

DISCLOSURES

The authors certify that they have no affiliations with or involvement in any organization or entity with any financial interest (such as honoraria; educational grants; participation in speakers' bureaus; membership, employment, consultancies, stock ownership, or other equity interest; and expert testimony or patent-licensing arrangements), or non-financial interest (such as personal or professional relationships, affiliations, knowledge or beliefs) in the subject matter or materials discussed in this manuscript.

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