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Original Article

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Using light and melatonin in the management of New Zealand White rabbits

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Abstract

Lighting system is a stimulant for reproduction in some species (Horses) and an inhibitor for others (Sheep). This study started on September 1st and planned to study the effects of different lighting regimes and melatonin treatment on the receptivity and performance of 78 (60-does and 18-bucks) New Zealand White rabbits, which were reared in a private Rabbitary in Menuofia Governorate, Egypt. These rabbits were randomly assigned to six treatment groups of 10 does and three bucks for each (8, 10, 12, 14 and 16 hours light (HL) and melatonin-treated). Ejaculate traits, sexual activity of bucks, sexual receptivity and reproductive performance of does were recorded. Results revealed that exposure of rabbits to long photoperiods (14 and 16HL) or treatment with melatonin improved the quantity and quality of ejaculate traits and buck sexual activity. Moreover, does sexual receptivity, feed intake, litter size and weight at birth and weaning were increased by long photoperiods (14 and 16HL) or treatment with melatonin. On the other hand, gestation period and pre-weaning mortality rate were decreased. It can be concluded that application of long photoperiods is beneficial to rabbit producers and 14HL:10 hours dark is optimal for satisfying the biological requirements of the rabbits. Finally, the light schedules can be used for biostimulation instead of melatonin.

Keywords: Light schedules, Melatonin, New Zealand White rabbits, Sexual receptivity.

Introduction

There are several environmental factors affecting animal welfare under intensive breeding conditions, such as lighting, temperature, gases, type of floor and cage enrichment. Most of mammals, which live in temperate climates, have their reproduction following a seasonal pattern that is often under photoperiodic control. Such patterns have evolved so that animals give birth during periods when environmental conditions are favorable to maximize the chance for young animals to survive. Photoperiod plays an important role in the reproduction of female animals. It is a well known fact that the reproduction period of wild rabbit starts with the elongation of light period, in spring, while it stops with the increase of the dark hours within the day (Lebas *et al.*, 1986). Rabbit productivity exhibits a seasonal pattern; it is much higher from September to June each year in the north of Egypt (Menuofia Governorate) with a peak in May and June. Some of these variations could be explained by reproductive performance, controlled by annual photoperiodic and ambient temperature changes.

In European latitude, the maximum breeding activity and most of the pregnancies occur between February and early August, with a peak in May when day length is increasing (Boyd, 1985; Theau-Clément *et al.*, 1998). In Europe, commercial rabbit producers adopted a 16 hours light (HL) : 8 hours darkness (HD) constant lighting schedule to minimize the negative effect of decreasing day length periods on reproduction (Alvarino and Ubilla, 1993). Although the amplitude of annual photoperiodic changes is smaller in the tropics, it may reach as long as 3 hours.

Using biostimulation techniques to improve reproductive efficiency, as the foreseeable evolution of the regulations on the use of exogenous hormones has led to study alternative methods for the improvement of sexual receptivity of rabbits and consequently their productivity, has increased recently (Theau-Clément and Boiti, 1998).

One of the biostimulation methods most frequently investigated is the use of lighting regimes. Lighting schedules, which are easy to apply and of low cost, will be more efficient in rabbit production. Moreover, lighting programs are widely used in avian species. Supplemental lighting programs produced positive effects on female reproductive precocity, kindling rate, and litter size (number of kits/birth) at birth under tropical conditions in Nigeria and Guadeloupe (Berepubo *et al.*, 1993; Depres *et al.*, 1996). Moreover, in seasonally breeding Syrian hamsters, day length influenced the copulatory behavior as males ceased to display ejaculatory behavior after several weeks from exposure to short day lengths (Powers *et al.*, 1989; Miernicki *et al.*, 1990).

Studies approaching photoperiod manipulation in rabbit farms have generally shown a significant improvement in receptivity and fertility of does when the daylight length was artificially increased (Harris *et al.*, 1982; Boyd, 1986). Optimizing semen production is of great importance for breeding programs, especially when artificial insemination

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(AI) is used. Therefore, ejaculates with a high number and good quality spermatozoa are the most important goals for rabbit AI centers. Unfortunately, semen characters vary during the life of rabbits. Both semen production and sperm quality can differ depending on individual characteristics, environmental and managemental factors.

Environmental conditions exert their influence on reproductive performance of domestic rabbits by variations in daylight length, air temperature and relative humidity (Hudson *et al*., 1994; Marai *et al*., 2002). Generally, reproductive performance of male rabbits is enhanced under long day light (Marai *et al*., 2002). The important photo-dependent hormone is the pineal melatonin, which plays an important role in the neuroendocrine control of reproductive cycle. Melatonin secretion is higher in dark (short day) period and lower in light (long day) period (Boyd, 1986; Bonanno *et al*., 2000). No information is available about effect of melatonin treatment on rabbit's performance. The objectives of this experiment were to examine the effect of light and melatonin treatments on the receptivity and performance of New Zealand White rabbits.

Materials and Methods

Animal management and treatment:

The present study was planned to study the effect of different lighting regimes and melatonin treatment on the receptivity and performance of New Zealand White rabbits reared in a private rabbitary in Menuofia Governorate, Egypt. The experimental work started on September 1st until the end of December. A total number of 78 New Zealand White rabbits (60-does and 18-bucks, with an average age of 9 months) were used in this study. These rabbits were randomly assigned to six treatment groups of 10 does and three bucks each. The rabbits in the first group were exposed to 8 hours light (HL) and 16 hours darkness (HD) (8HL:16HD), the rabbits in the second group were exposed to 10HL:14HD, the rabbits in the third group were exposed to 12HL:12HD, the rabbits in the fourth group were exposed to 14HL:10HD, the rabbits in the fifth group were exposed to 16HL:8HD and the rabbits in the sixth group were treated with melatonin 3mg/day per os (VIVA-MAX 3) (Amoun Co., Cairo) for two weeks and exposed to 11 HL (natural light). For all groups, the duration of the treatment was 60 days. Mating of females was carried out naturally using bucks from the same group (each doe mated twice by different bucks, to maximize the conception rate and litter size) at the end of treatment (Mousa-Balabel, 2004). The rabbits in all groups were offered a commercial pelleted diet (18% crude protein, 12% crude fiber, 2.8% ether extract and 2600 kcal DE/kg diet digestible energy) and clean water *ad libitum*. The rabbits were housed in a room of the rabbitary, which was divided into two parts: one for melatonin-treated group, which was exposed to 11 HL (natural daylight) via windows, and the other part of the room was supplied with fluorescent light lamps and black curtains on the windows to control the light hours for light-treated groups (the other five groups).

The Rabbitary was naturally ventilated through windows and was provided with automatically-controlled side exhaustion fans. The rabbits were individually housed in galvanized wire cages (50 x 55 x 39cm) provided with a feeder and automatic nipple drinker. All rabbits were kept under the same hygienic and managerial conditions (room temperature varied from 25 to 27.5°C for all groups). Supplementary lighting was provided by fluorescent lamps at the beginning of the dark period, while decreasing the light duration (as in group one and two) was achieved by the use of black curtains on the inner side of the room walls. The natural daylight was estimated daily by the difference between sunrise and sunset times. Intensity of light was estimated by a lux-meter fixed on the cages either under natural or artificial lighting and its value was approximately 20 lux.

***Measurements*:**

Ejaculate traits, sexual activity of bucks (reaction time; the time elapsed from exposure of buck to receptive doe till ejaculation in seconds), sexual receptivity (receptive doe takes a lordosis position in the presence of buck with red or purple color of the vulva) and reproductive performance of does were recorded. Feed intake and water consumption were estimated individually daily during the experimental period.

Each time, feed intake was measured by subtracting the residuals of feed from that offered for each animal. Water intake was estimated by measuring the difference in the water volume in the crocks. Bucks were trained for semen collection using one of several female teasers and an artificial vagina. Two ejaculates per week from each buck were collected for two months starting from day one after termination of the treatment to get the maximum effect of the light treatment using an artificial vagina following the procedure described by Boussit (1994). Ejaculate volume, sperm volume, sperm concentration, live spermatozoa, sperm motility and morphologic abnormalities (head, midpiece and tail defects) were evaluated according to Roca *et al*., (2000).

Statistical analysis:

Data were collected, arranged, summarized and analyzed using the general linear model procedures of the SAS, Institute INC (1985).

Results

In Table 1, the results of different light regimes and melatonin treatment on semen traits and reaction time for New Zealand White rabbit bucks are presented. The ejaculate and semen volumes for bucks, which were exposed daily to photoperiods of 8, 10 and 12HL were less than and differed significantly ($P<0.05$) from those exposed to 14 and 16HL and those treated with melatonin. The results showed that the bucks exposed to 14 and 16HL and those treated with melatonin had higher sperm concentrations and live spermatozoa percentage and differed significantly ($P<0.05$) from other treatment groups. There was a significant difference ($P<0.05$) in the abnormality of sperm cells for bucks exposed to different treatments.

The lowest percentage of abnormality in the sperm cells was for bucks treated with melatonin while, the highest one for those exposed to 8HL. The percent of primary abnormality in the sperm cells for other treatment groups was of the order 16HL < 14HL < 12HL < 10HL. There were variations in progressive sperm motility for bucks exposed to the different treatments. The results showed that bucks exposed to 14 and 16HL and those treated with melatonin had progressive sperm motility higher ($P<0.05$) than the other treatments. The reaction time significantly ($P<0.05$) decreased in the group treated with melatonin (26 sec.), in the group of bucks exposed to 16HL (28 sec.) and in the group of bucks exposed to 14HL (32 sec.). Meanwhile, it was 59, 57 and 57 sec. for bucks exposed to 8, 10 and 12HL, respectively. Data on sexual receptivity of does expressed by the color of vulva are presented in Table 2.

The melatonin treatment and increased photoperiod had a clear influence ($P<0.05$) on does' sexual receptivity. For both lighting schedules 14, 16HL, and melatonin treatment, most of the females had (80, 90 and 90% respectively) red and purple vulvas color, whereas using the light schedules 8, 10 and 12HL, percentages of does with red vulva significantly decreased (20, 40 and 50% respectively) in favor to those having pale or pink vulvas.

Table 1: Effect of different light regimes and melatonin treatment on semen traits and reaction time of New Zealand White rabbit bucks (Mean ± SE).

Treatment	8HL:16HD	10HL:14HD	12HL:12HD	14HL:10HD	16HL:8HD	Melatonin
Ejaculate volume (ml)	0.8 ± 0.043^{b}	0.8 ± 0.068^{b}	0.8 ± 0.021^{b}	0.98 ± 0.092^{a}	1.02 ± 0.068^{a}	1.08 ± 0.021^{a}
Semen Volume (ml)	0.72 ± 0.006^{b}	0.74 ± 0.008^{b}	0.75 ± 0.006^{b}	0.81 ± 0.005^{a}	0.84 ± 0.006^{a}	0.86 ± 0.008^{a}
Sperm concentration (10^{6}/ml)	252 ± 0.065^{c}	274 ± 0.026^{c}	276 ± 0.045^{c}	319 ± 0.009^{b}	324 ± 0.026^{b}	336 ± 0.045^{a}
Live Spermatozoa %	67 ± 0.037^{b}	68 ± 0.013^{b}	69 ± 0.055^{b}	73 ± 0.076^{a}	73 ± 0.013^{a}	74 ± 0.055^{a}
Abnormality %	9.8 ± 0.051^{a}	9.2 ± 0.029^{a}	8.9 ± 0.077^{a}	5.8 ± 0.012^{b}	5.9 ± 0.029^{b}	5.5 ± 0.077^{b}
progressive sperm motility %	67 ± 0.026^{b}	68 ± 0.049^{b}	68 ± 0.011^{b}	72 ± 0.069^{a}	73 ± 0.049^{a}	73 ± 0.011^{a}
Reaction time (sec)	59 ± 0.065^{a}	57 ± 0.063^{a}	57 ± 0.085^{a}	32 ± 0.012^{b}	28 ± 0.041^{c}	26 ± 0.018^{c}

a,b,c. Means bearing different superscripts in the same row differ significantly ($P<0.05$).

Table 2: Effect of different light regimes and melatonin treatment on receptivity and performance of New Zealand White rabbit does (Mean ± SE).

Treatment		8HL:16HD	10HL:14HD	12HL:12HD	14HL:10HD	16HL:8HD	Melatonin
Receptivity	Pale and Pink (%)	80 ± 0.010^{a}	60 ± 0.075^{b}	50 ± 0.079^{c}	20 ± 0.063^{d}	10 ± 0.081^{e}	10 ± 0.079^{e}
	Red and purple (%)	20 ± 0.071^{e}	40 ± 0.079^{d}	50 ± 0.076^{c}	80 ± 0.069^{b}	90 ± 0.088^{a}	90 ± 0.070^{a}
Conception Rate (%)		40 ± 0.051^{f}	50 ± 0.036^{e}	60 ± 0.078^{d}	70 ± 0.080^{c}	90 ± 0.087^{b}	100 ± 0.044^{a}
Feed intake (g/day)		140 ± 0.016^{c}	141 ± 0.026^{c}	145 ± 0.034^{c}	160 ± 0.030^{b}	172 ± 0.044^{a}	179 ± 0.016^{a}
Water consumption (ml/day)		260 ± 0.051^{b}	265 ± 0.027^{b}	270 ± 0.055^{b}	308 ± 0.038^{a}	315 ± 0.013^{a}	320 ± 0.051^{a}
Gestation Period (days)		31.2 ± 0.008^{b}	31.9 ± 0.012^{b}	31 ± 0.008^{b}	29 ± 0.012^{a}	29 ± 0.008^{a}	29 ± 0.012^{a}
Litter size (No. of kits)		3.1 ± 0.022^{d}	4.0 ± 0.030^{d}	4.5 ± 0.022^{d}	6.2 ± 0.030^{c}	7.2 ± 0.022^{b}	8.5 ± 0.030^{a}
Kit weight at birth (g)		45 ± 0.021^{b}	48 ± 0.030^{b}	47 ± 0.021^{b}	58 ± 0.030^{a}	58 ± 0.030^{a}	60 ± 0.009^{a}
Kit weight at weaning (g)		410 ± 0.048^{b}	415 ± 0.036^{b}	412 ± 0.048^{b}	455 ± 0.036^{a}	$460\pm.048^{a}$	460 ± 0.036^{a}
Pre-weaning mortality %		25.8 ± 0.052^{a}	20 ± 0.014^{b}	17.7 ± 0.052^{c}	12.9 ± 0.014^{d}	11.1 ± 0.014^{d}	9.4 ± 0.033^{e}

a,b,c,d,e,f. Means bearing different superscripts in the same row differ significantly ($P<0.05$).

It is obvious that conception rates were significantly (P<0.05) influenced by using light schedules and melatonin treatment. The results showed that does treated with light regimes, 8, 10 and 12HL had lower conception rates in comparison with those obtained from does treated with melatonin, 14 and 16HL (100, 90 and 70%, respectively).

Feed intake and water consumption increased by increasing photoperiod (14 and 16HL) and using melatonin treatment when compared to other treatments (8, 10 and 12HL). On the other hand, the gestation period was decreased by using long photoperiods (14 and 16HL) and melatonin treatment. Litter size obtained from does subjected to photoperiods 8, 10 and 12HL was lower than those subjected to photoperiods 14 and 16HL and melatonin treatment.

The kits weight at birth and weaning was affected by light schedules and melatonin treatment. Results showed that kits weight at birth and weaning obtained from does exposed to light schedules 8,10 and 12HL was lower than, and differed significantly (P<0.05) from, those exposed to 14, 16HL and melatonin-treated. Pre-weaning mortality rate was decreased by increasing photoperiods (14 and 16HL) and by using melatonin in comparison with short photoperiods 8, 10 and 12HL.

Discussion

Length of daylight seemed to affect most reproductive traits of both sexes of rabbits. Particularly, exposure of rabbits to long daylight often showed favorable effects on their reproductive traits.

Treatment with melatonin and long photoperiods (14 and 16HL) increased the ejaculate and semen volumes, sperm concentrations, percentage of live spermatozoa and mass motility. This may be explained by light length affecting the hypothalamus-pituitary axis and consequently hormonal release and spermatozoa production (qualitative and quantitative aspects) (Theau-Clément *et al*., 1994) and this could be responsible for the significant increase in sperm output. Therefore, the exposure of bucks to one of these treatments would be of great benefit to ensure the production of mature spermatozoa and acceptable sperm quality.

The lowest abnormality percentages were observed in the semen for bucks exposed to treatment with melatonin and long photoperiods (14 and 16HL). These abnormalities consequent to treatment have important consequences on quality of the spermatozoa and subsequent fertility (Luthman and Slyter, 1986).

These results are in agreement with those reported by Theau-Clément *et al*. (1994) who recorded that exposure of rabbits to long days (16HL:8HD) improved the quantity and quality of spermatozoa present in the ejaculates in comparison to those collected in rabbits exposed to short day light (8HL:16HD). Contrary to the observations of Mahrose *et al*. (2010) who reported that all semen characters were significantly improved with short photoperiod (12HL:12HD), except dead spermatozoa percentage that was significantly declined with increasing photoperiod to 16 hrs/day. This difference may be attributed to different ages of rabbits he used (6 months).

As the ejaculate volume (gel-free semen and gel fraction together) varies seasonally, the seasonal variation in gel-free semen volume is related to changes in gel volume, which was lowest during summer. This could indicate that, during summer, there is a minor gelatinization of seminal plasma, probably due to the adverse effect of high ambient temperature. The fact that motility index was lowest during summer also suggests that the seasonal variations could be associated with changes in the environmental temperature (Marai *et al*., 2002).

The reaction time decreased by the treatment with melatonin and increased photoperiod (14 and 16HL) leading to improvement in the sexual activity of bucks. This may be attributed to the improvement of the testicular size and hormonal release especially testosterone in bucks reared under long day light. These results coincided with those obtained by Boyd (1986) who reported that the reproductive regression in wild rabbits can be induced by a reduction in the day length,

The sexual activity of rabbits, in natural conditions, is maximal during the long-day seasons. By artificially increasing the daylight length in rabbit farms does sexual receptivity and fertility may be improved.

Continuous lighting programs of 14 or 16HL per day all year round seem to have a positive effect on does reproduction (Uzcategui and Johnston, 1992) . The color of the vulva is an indicator of receptivity, which is determined by the serum estrogen levels secreted by the growing ovarian follicles (Castellini, 1996). Red and purple vulva colors are believed to correspond to maximal receptivity and fertility in lactating does (Theau-Clément and Roustan, 1992) while, pale and pink are believed to correspond to lower receptivity. In this study, under light schedules 8, 10 and 12HL, only a very small percentage of females exhibited red and purple vulvas.

Therefore, using melatonin or increasing the photoperiod to 14 and 16HL associated with a higher sexual stimulation known to be naturally experienced by rabbits during long day seasons (Boyd, 1986) significantly improved sexual receptivity of the does in comparison with decreased lighting programs. The highest sexual receptivity of does associated with high conception rates of those does. The light stimulation significantly increased the conception rate. Similar results were reported by Theau-Clément *et al*., (1990); Mirabito *et al*., (1994); Maertens and Luzi

(1995). Feed intake and water consumption were significantly lower for rabbits kept under photoperiods 8, 10 and 12HL than for those treated with melatonin and increased photoperiod (14 and 16HL), which can be explained by light stimulation to the pineal function leading to increase in cortisol hormone in does exposed to long daylight through activation of the hypothalamic pituitary-adrenal axis and the consequent increase of plasma glucocorticoid concentrations (Christison and Johnson, 1972).

On the contrary, gestation period was found to be shorter in does kept under the light regimes (14 and 16HL) and in those treated with melatonin than other treatment groups (8, 10 and 12 HL). This may be attributed to the increase in the litter size and the larger weight of the litters for does in these groups.

Litter size obtained from does treated with melatonin and from those subjected to long photoperiods (14 and 16HL) was significantly larger than those subjected to photoperiods 8, 10 and 12HL. This can be explained by the number of ovulations is slightly higher in does subjected to long light.

Significant differences in the kit`s weight at birth and weaning were observed. The largest kit`s weight at birth and weaning was recorded in kits for does treated by melatonin and for those exposed to long day light (14 and 16HL). These results are in agreement with those recorded by Mirabito *et al*. (1994) who observed that the litter weight as well as the individual weight at the age of 3 weeks was slightly decreased under a 8HL:16HD photoperiod. This may be due to exposure to long photoperiod increaseing the milk production of does because of the light stimulation modifying the duration of nursing events.

The frequency of multiple nursing and the number of nursing events per day increased when the dark period was shorter (8 hrs instead of 16 hrs) (Gerencsér *et al*., 2008). There were significant differences in the kits pre-weaning mortalities; using melatonin and long photoperiods (14 and 16 HL) decreased the pre-weaning mortality rate among kits for those does when compared to other treatment groups (8, 10 and 12 HL). This can be explained by the improved nursing behavior for does kept in these groups (Gerencsér *et al*., 2008) in addition to the remarked increase in the kits weight at birth.

From the results of the present work, it can be concluded that application of long light schedules improved the reproductive performance of the rabbits. The lighting schedule; 14HL:10HD is optimal for satisfying the biological requirements of the rabbits. Melatonin treatment and light schedules, 14 and 16HL are believed to improve ejaculate parameters of male rabbits, receptivity of does, feed consumption, litter size and kit`s weight at birth and weaning. Meanwhile, it lowered the pre-weaning mortality rate among kits. Finally, light regimes can be used as an alternative biostimulant for melatonin.

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