Research Article

Effects of Ultraviolet-B Radiation on Behaviour, Reproductive Performance, Serum Vitamin D Status and Gene Expression in Female Rabbits

Asmaa S. Mohammed*, Ahmed F. Abou-Elnaga, Ahmed I. Ateya, Mohammed M. Fouda, Ragab A. Darwish, Usama A. Abou-Ismail

Department of Husbandry and Development of Animal Wealth, Faculty of Veterinary Medicine, Mansoura University, Gomhoria St., Mansoura, P.O. Box 35516, Egypt.

Abstract | This experiment aimed to investigate the effect of exposure to different durations of UV-B radiation on behaviour, reproductive performance, serum vitamin D levels, cortisol levels and expression pattern of *follicle stimulating hormone receptor* (*FSHR*) and *estrogen receptor alpha* (*ER-α*) genes in female rabbits. A total of 84 Nulliparous Blanc de Bouscat does were randomly allocated into one of four experimental groups (non-UVB, 12-h UVB, 8-h UVB and 4-h UVB) according to the duration of exposure to UVB radiation (n = 21 for each group). Behavioural patterns of rabbits were monitored for six consecutive weeks whereas, reproductive performance, expression profile of *FSHR* and *ER-α* genes and levels of serum 25-hydroxy vitamin D and cortisol were evaluated at the end of the exposure period. The results showed that the exposure of female rabbits to prolonged durations of UVB radiation (8 and 12 hours) reduced the level of inactive behaviours and bar chewing, and improved maintenance and exploratory behaviours. Similarly, the exposure to long durations of UVB radiation elevated serum 25-hydroxy vitamin-D to safe levels and decreased mortality rate of kits. Furthermore, the exposure to 8 hours of UVB radiations in particular improved reproductive indices including litter weight at weaning and Kits' weight gain from birth to weaning, and up-regulated the relative expression levels of *FSHR* and *ER-α* genes. Therefore, based on the multiple measures approach used to evaluate the effect of UVB light on behaviour, reproductive and health status of rabbits, it could be suggested that eight hours of UVB radiation appears the most optimal duration for indoor housed female rabbits.

Keywords | Behaviour, Gene expression, Rabbits, UVB radiation

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***Correspondence** | Asmaa S. Mohammed, Department of Husbandry and Development of Animal Wealth, Faculty of Veterinary Medicine, Mansoura University, Gomhoria St., Mansoura, P.O. Box 35516, Egypt; **Email:** asmaasaad723@gmail.com
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INTRODUCTION

Vitamin D is considered an essential part of nutrition for humans, mammals and poultry to maintain good health. It functions to maintain serum calcium concentrations within the normal range (Browning and Coweison, 2014). The biological actions of vitamin D are mediated through its receptor (VDR). VDR is known to be a member of steroid/thyroid nuclear hormone receptor superfamily (Johnson and DeLuca, 2001). VDR has been involved not only in calcium regulation issues such as skeleton, intestines and parathyroid gland, but also in other reproductive organs as uterus, placenta, ovary (granulosa cells), testis, pituitary gland and hypothalamus (Kinuta et al., 2000). This divergent expression of VDR implies a potential role of vitamin D in female reproduction. Sun light is widely known to be the greatest source of UV radiation; UV light represents about 10 % of

electromagnetic radiation emitted from the sun. However, man-made UV sources have been emerged over the years including several types of UV lamps, mercury vapour lamps and recently LED UVB bulbs (Martin and Hine, 2008). One of the crucial benefits of UVB radiation, is enabling vitamin D metabolism, where insufficient exposure can result in vitamin D deficiency. In humans, vitamin D deficiency during pregnancy has been related to increased risk of recurrent pregnancy loss, pregnancy diabetes and preeclampsia (Alzaim and Wood, 2013). Humans, reptiles, many birds and small mammals have been reported to have the ability to synthesize vitamin D in the epidermis in response to UVB exposure. Rabbits have been reported to produce vitamin D metabolites in corneal epithelium following UVB exposure (Lin et al., 2012). In indoor housed mammals including rabbits, UVB light exposure has been widely substituted with dietary supplementation of vitamin D. However, dietary supplementation may present the risk of vitamin D toxicity in contrast to UVB exposure (Antwis et al., 2009). So far, relatively few studies have been conducted to demonstrate the effectiveness of UV-B radiation from natural or artificial sources in increasing serum 25-hydroxy vitamin D levels in juvenile and adult rabbits. Moreover, no data is available on the most optimum duration of UVB radiation to ensure benefits and how growth and reproduction could be affected. Therefore, the purpose of the current study was to investigate the effect of three durations of UVB light/day (12, 8 and 4 hours) on behaviour, serum 25-hydroxy vitamin D status and cortisol level, reproductive performance and expression pattern of *ER-α* and *FSHR* genes in female rabbits.

MATERIALS AND METHODS

Ethics statement

The study protocol was approved by the Research Ethics Committee of the Faculty of Veterinary Medicine, Mansoura University, Egypt (Code Ph.D/34).

Animals and husbandry

The study was carried out using a total of 84 Nulliparous Blanc de Bouscat does (Oryctolagus cuniculus domesticus), which were obtained from a private rabbit farm. Does were obtained with an average initial age of 5 months, and they were used for service for the first time at the age of 6 months. They were ranging in weight from 3 to 3.5 kg. General room lighting was provided with non UVB producing LED bulbs of 30 lux light intensity and a 16L: 8D light dark cycle was maintained. Does were individually housed in standard wire battery cages (50 W x 60 D x 30 H), each equipped with a feeder, nipple drinker and internally attached nest box. The rooms temperature was measured daily and maintained consistent throughout the whole study with an average range of 24 to 27°C. All does were fed a standard commercial complete pelleted diet (CFD) containing 500 IU/ kg of vitamin D3 and formulated to satisfy the nutritional requirements for pregnancy and lactation according to the recommended in NRC (1977). Moreover, female rabbits were fed ad-libitum during late pregnancy (25-33 day) and throughout the whole lactation period, while all other does were feed-restricted, receiving 140 to 150 g/d, and tap water was always available.

Experimental design

Rabbits were acclimated for 7 days prior to the start of the study. They were randomly assigned to four experimental groups of 21 each according to the duration of UVB radiation they will be exposed to. Rabbits in the control group were maintained under the typical white LED lighting (non-UVB) and were not exposed to supplemental UVB light. Rabbits in 12-h, 8-h and 4-h UVB groups were exposed to supplemental UVB lighting for 12 hours, 8 hours and 4 hours per day, respectively. General room lighting was maintained using non UVB producing white LED bulbs (Philips E27 Star LED Bulb, 12 watt). While UVB radiation was provided using 2 centrally placed 26 W UVB 5.0 compact fluorescent lamps (Changzhou Jinxu Special Lighting Co., Ltd) above each battery. The 2 lamps were placed at a distance of 30 cm from the rabbit head, and 50 cm from cage floor. The quantity of UVB radiation and irradiance was measured in microwatts per centimeter square (mW /cm^2) using a digital UVB meter (Solarmeter® Model 6.2 Sensitive UVB Meter). UVB meter was placed under each lamp at the level of rabbit's head and between the lamps as well. UVB meter was used weekly about 3 hours after the lamps were turned on to ensure that UVB radiation levels is not less than 20 mW/cm^2 (Watson et al., 2019).

Behavioural observations

Behavioural categories of rabbits were always sampled by the same observer. Rabbits behaviour was recorded by direct observation using instantaneous scan sampling technique (10 sec). The recordings were carried out in two periods (9:00 h and 17:00 h), two hours each, over 4 consecutive days each week for a 6 weeks period. The observer entered the room 10 minutes before the scheduled observation time started. The observer then walked slowly into the visual field of animals and recordings were made as far as possible from animals to minimize disturbance. Following preliminary observation of rabbits behaviour, an ethogram based on (Gunn and Morton, 1993) was developed (Table 1).

Reproductive performance traits

One month after does in treatment groups were exposed to UVB radiation, does were naturally mated with fertile Blanc de Bouscat bucks (sex ratio was 1:5). Litter size at birth (LSB) and weaning (LSW), litter weight at birth (LWB) and weaning (LWW), litter weight gains from birth

to weaning (LWG), kindling rate, viability rate, gestation length and pre-weaning mortality were recorded for all groups according to the recommendations of international rabbit reproduction group (IRRG) (IRRG, 2005).

Serum collection and analysis

Three blood samples were collected, the first collection was carried out after initial rabbit's acclimation period, the second was at day 15 of pregnancy; 5 days after pregnancy was confirmed, while the third was scheduled at day 20 of lactation. Blood samples were collected from lateral marginal ear vein from 15 does in each experimental treatment for evaluation of serum 25-hydroxy vitamin D status and serum cortisol levels. A 2 ml blood sample was collected from each rabbit using a 20-gauge needle in a red- topped Vacutainer tubes with no anticoagulant. Specimens were allowed to clot at room temperature and then stored at - 4˚C for 12 hours until shipped to a veterinary diagnostic laboratory for analysis.

Tissue collection

After weaning, 10 randomly selected does from each group were euthanized by intravenous injection of sodium pentobarbital (100 mg/kg), tissue samples were collected from right ovary for *follicle stimulating hormone receptor* (*FSHR*) and *estrogen receptor alpha* (*ER-α*) genes expression. Tissue samples were then placed in sterilized Eppendorf tubes containing RNA later solution for stabilizing cellular RNA in tissue samples and inhibiting *RNases* activity and then stored at –80˚C for total mRNA extraction.

RNA extraction and real-time PCR

Total RNA was extracted from ovarian tissue samples of experimental rabbits according to miRNeasy Mini Kit instruction guidelines (Cat no 217004, Qiagen, Germany). The reverse transcription of cDNA from the extracted ovarian RNA was completed using SensiFAST™ cDNA synthesis kit (Bioline, United Kingdom) according to the constructer's manual. Consequently, quantitative real-time PCR (qRT-PCR) was carried out via Stratagene MX3005P real-time PCR (Agilent Technologies, CA, USA) by the use of QuantiTect SYBR Green PCR kits (Qiagen, Heidelberg, Germany) and specific primers for each gene are depicted in Table 2. The thermal cycling conditions are illustrated in Table 3. Melting curves were created following qRT-PCR to identify specific amplification of each target gene of interest. The relative expression of the gene in each sample was normalized in comparison to GAPDH (Glyceraldehyde-3-Phosphate Dehydrogenase) gene and calculated according to the 2-ΔΔCt method (Yuan et al., 2006).

Data analysis

Data were tested for distribution normality, linearity and homogeneity of variance. Statistical analyses were performed using SPSS software ver. 23.0 (IBM SPSS Statistics for Windows, Version 23.0. Armonk, NY: IBM Corp). To investigate the effect of treatment on behavioural expression, reproductive performance parameters, serum vitamin D and cortisol levels and expression pattern of *FSHR* and *ER-α* genes, One-way ANOVA was used. While kindling rate of the does was compared by means of Chi-square test. Data are reported as means ± SEM and the Duncan's multiple range test was used as a post hoc test to determine the nature of the significant effects. Differences were considered to be significant at the level of $P \leq 0.05$.

Table 1: Ethogram of behavioural elements recorded.

Behaviour	Definition
Feeding	Rabbit is eating food pellets from the hopper.
Drinking	Drinking of water directly from water nipples.
Self-grooming	Licking own coat with sweeping movements of the head or licking forepaws and pulling them over the face and ears.
Rearing up	Sitting up on hindlimbs while front paws are lifted from the floor with body stretching upwards.
Sniffing	Sniffing items of the environment (floor, wall, hopper, bars and roof of the cage) or sniffing the air with nose.
Stretching	Front legs are stretched out in front while the rabbit is standing.
Standing	Standing supporting weight on four limbs, with no apparent activity.
Lying down	Lying with limbs under body, trunk is resting on the ground, hindlimbs tucked under body and forelimbs lying under body or stretched forward from the body.
Sitting	Body against floor but still supported by fore and hind limbs.
Bar chewing	Biting the cage bars or the floor.

Table 2: Forward and reverse primer sequences, accession number and length of PCR product of target genes.

Gene	Accession number	Primer sequence	Product size	Reference
GAPDH	NC-013676.1	F: 5′-TGTTTGTGATGGGCGTGAA-3′ R: 5′-CCTCCACAATGCCGAAGT-3′	150 bp	(Lan *et al.*, 2014)

FSHR	AY429104.1	F: 5′-GAGGAATGCCATTGA ACTGAGG-3′ R: 5′-AAGGTTGGAGAACACATCTG-3′	150 bp	(Lan *et al.*, 2014)
***ER*-α**	XM_002714947	F: 5′-CCTCCTCATCCTCTCCCACA-3′ R: 5′-AGCATCTCCAGCAACAGGTC-3′	111 bp	(Han *et al.*, 2014)

[a]*GAPDH*= glyceraldehyde 3 Phosphate Dehydrogenase, [b]*FSHR*= follicle stimulating hormone receptor, [c]*ER-α* = estrogen receptor alpha.

Table 3: Reverse transcription and real-time PCR program for *ER-α*, *FSHR* and *GAPDH* genes.

Gene	Reverse transcription	Initial denaturation	Amplification (40 cycles)			Dissociation curve (1 cycle)		
			Secondary denaturation	Annealing	Extension	Secondary denaturation	Annealing	Extension
GAPDH	50°C /30 m	95°C /10 m	95°C /15 s	60°C /30 s	72°C /30 s	95°C /1 m	60°C /1 m	72°C /1 m
FSHR	50°C /30 m	95°C /10 m	95°C /15 s	57°C /30 s	72°C /30 s	95°C /1 m	57°C /1 m	72°C /1 m
ER-α	50°C /30 m	95°C /10 m	95°C /15 s	61°C /30 s	72°C /30 s	95°C /1 m	61°C /1 m	72°C /1 m

[a]*GAPDH*= glyceraldehyde-3-Phosphate Dehydrogenase, [b]*FSHR*= follicle stimulating hormone receptor, [c]*ER-α* = estrogen receptor alpha.

RESULTS AND DISCUSSION

Effect of UVB radiation on behavior expression

There was an effect to the exposure to different durations of UVB radiation on the levels of behavioural patterns of rabbits (Table 4). Rabbits experienced 12-h UVB exhibited the highest levels of feeding (P=0.05) compared to those in either non-UVB control or 4-h UVB group, and the highest levels of drinking compared to non-UVB control group (P=0.02). Moreover, levels of stretching behaviour were higher in 12-h UVB and 8-h UVB groups than in non-UVB control group (P=0.008, P=0.02, respectively) and 4-h UVB group (P=0.01, P=0.04, respectively). Furthermore, rabbits in non-UVB group exhibited the highest levels of inactivity including sitting (P=0.005, P=0.04, P=0.001, respectively) and lying down (P=0.001) compared to those in 12-h, 8-h and 4-h UVB groups. Additionally, the highest levels of maintenance (grooming) and exploration (sniffing) were observed in 12-h, 8-h and 4-h UVB groups compared to non-UVB control group (P=0.001). In particular, rabbits experienced 4 hours of UVB light performed the highest levels of rearing up compared to those experienced no UVB light in the control group (P=0.01). The highest frequencies of bar chewing were recorded in rabbits of non-UVB group (P=0.01, P=0.005, respectively) and 4-h UVB group (P=0.003, P=0.001, respectively) compared to those in 12-h and 8-h UVB groups. However, levels of standing behaviour did not differ between the experimental groups.

Table 4: Mean % scan sampling (±SEM) of behavioural patterns of rabbits in different experimental groups.

Behavioural pattern	non-UVB	12-h UVB	8-h UVB	4-h UVB
Feeding	4.18 ± 0.50^{B}	5.86 ± 0.75^{A}	4.52 ± 0.64^{AB}	4.02 ± 0.48^{B}
Drinking	0.72 ± 0.14^{B}	1.39 ± 0.21^{A}	1.20 ± 0.26^{AB}	1.19 ± 0.18^{AB}
Standing	1.70 ± 0.25	1.37 ± 0.31	2.00 ± 0.29	1.66 ± 0.25
Stretching	0.10 ± 0.05^{b}	0.38 ± 0.08^{a}	0.33 ± 0.09^{a}	0.13 ± 0.04^{b}
Sitting	9.90 ± 0.92^{a}	6.95 ± 0.59^{b}	7.70 ± 0.74^{b}	5.72 ± 0.62^{b}
Lying down	35.08 ± 1.05^{a}	28.85 ± 1.07^{b}	30.33 ± 1.04^{b}	29.60 ± 0.87^{b}
Grooming	6.54 ± 0.61^{b}	12.68 ± 0.80^{a}	11.33 ± 0.83^{a}	13.08 ± 0.78^{a}
Sniffing	0.72 ± 0.15^{b}	2.70 ± 0.41^{a}	2.53 ± 0.39^{a}	2.04 ± 0.24^{a}
Rearing up	0.02 ± 0.02^{b}	0.08 ± 0.04^{ab}	0.10 ± 0.05^{ab}	0.18 ± 0.05^{a}
Bar chewing	1.00 ± 0.27^{a}	0.33 ± 0.09^{b}	0.22 ± 0.08^{b}	1.14 ± 0.23^{a}

[A, B] Means within the same row having different upper-case superscripts are significantly different at $P \leq 0.05$, [a, b] Means within the same row having different lower-case superscripts are significantly different at $P \leq 0.01$.

Effect of UVB radiation on reproductive and productive performance traits

There was an effect to the exposure of rabbits to the UVB radiation on several traits of their productive and reproductive profile (Table 5). LWB of rabbits in non-UVB group was lower than that of those in 12-h UVB group (P=0.01). While, LWW of rabbits in 8-h UVB group was higher when compared to those in either non-UVB control group, 12-h or 4-h UVB groups (P=0.002, P=0.001, P=0.001, respectively). LWG from birth to weaning was higher in 8-h UVB group compared to those in non-UVB control group (P=0.05). UVB radiation had a profound effect in reducing mortality rate of kits from birth to weaning as mortality rate of kits in non-UVB control group was higher than those in either 12-h, 8-h or 4-h UVB groups (P=0.003, P=0.003, P=0.01, respectively).

Moreover, kindling rate was higher in does kept in 12-h and 8-h UVB groups compared to those in non-UVB and 4-h UVB groups (P=0.003, P=0.02, respectively). However, LSB, LSW, viability rate and gestation period did not differ between experimental groups.

Effect of UVB radiation on serum biochemical analysis

Baseline levels of serum 25-hydroxy vitamin D taken after initial acclimation period did not differ among the experimental groups (P=0.7) (Figure 1). Whereas, levels at day 15 of pregnancy (Figure 2) and day 20 of lactation (Figure 3) were higher in does of 12-h, 8-h and 4-h of UVB light compared to those in non-UVB control group (P=0.001). Meanwhile, does in 12-h and 8-h UVB groups had higher levels of serum 25-hydroxy vitamin D than those in 4-h UVB light (P=0.001). On the other hand, serum levels of cortisol did not differ between experimental groups either at baseline levels, at day 15 of pregnancy or at day 20 of lactation.

Table 5: Mean (±SEM) of reproductive and productive performance traits of female rabbits in different experimental groups.

Traits	non-UVB	12-h UVB	8-h UVB	4-h UVB
Litter size at birth	9.00 ± 0.88	7.66 ± 0.33	7.33 ± 1.45	7.00 ± 0.57
Litter size at weaning	5.66 ± 0.33	7.00 ± 0.00	6.66 ± 1.20	6.00 ± 0.57
Litter weight at birth (g)	48.33 ± 2.04^{b}	57.50 ± 1.96^{a}	54.52 ± 3.63^{ab}	53.54 ± 2.45^{ab}
Litter weight at weaning (g)	430.58 ± 30.76^{b}	450.61 ± 23.40^{b}	554.25 ± 28.63^{a}	394.75 ± 24.61^{b}
Kits weight gain from birth to weaning (g)	373.13 ± 65.53^{B}	500.29 ± 80.17^{AB}	556.30 ± 91.09^{A}	380.24 ± 20.24^{AB}
Mortality rate (%)	37.11 ± 9.27^{a}	8.62 ± 4.33^{b}	9.14 ± 4.16^{b}	14.28 ± 4.33^{b}
Kindling rate (%)	85.70 ± 0.00^{b}	100.00 ± 0.00^{a}	100.00 ± 0.00^{a}	90.46 ± 4.76^{b}
Viability rate (%)	91.66 ± 8.33	95.83 ± 4.16	95.23 ± 4.76	95.23 ± 4.76
Gestation period	30.66 ± 0.33	30.33 ± 0.33	29.66 ± 0.66	31.66 ± 0.33

$^{A, B}$ Means within the same row having different upper-case superscripts are significantly different at $P\leq0.05$, $^{a, b}$ Means within the same row having different lower-case superscripts are significantly different at $P\leq0.01$. g= gram.

Effect of UVB radiation on expression pattern of reproductive genes

There was an effect to the exposure of female rabbits to UVB radiations on the relative expression levels of *FSHR* and *ER-α* genes. Female rabbits in the 8-h UVB group exhibited a significant up-regulation of *FSHR* gene expression in the ovary by 4.04 folds compared to those in either non-UVB control, 12-h or 4-h UVB groups (P=0.005, 0.05, 0.006, respectively) (Figure 4). Similarly, female rabbits in 12-h UVB group showed a significant up-regulation of *FSHR* gene expression in the ovary by 2.53 folds compared to those in non-UVB control group (P=0.05). Similarly, female rabbits in the 8-h UVB group showed a boosted up-regulation of *ER-α* gene expression by 5.15 folds compared to those in either non-UVB control group, 12-h or 4-h UVB groups (P=0.001) (Figure 5). Meanwhile, relative *ER-α* gene expression was up-regulated by 3.47 folds in 12-h UVB group compared to those in non-UVB control and 4-h UVB groups (P=0.001)

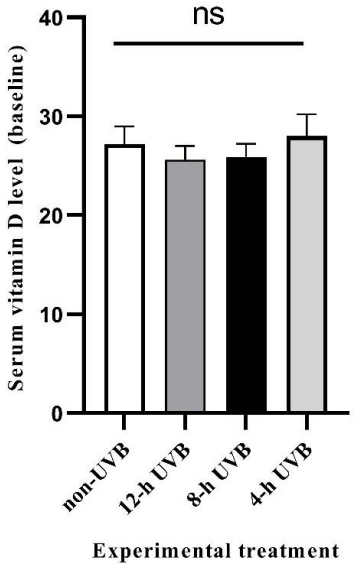

Figure 1: Effect of UVB radiation on serum 25-hydroxyvitamin D levels of does after initial acclmatation period.

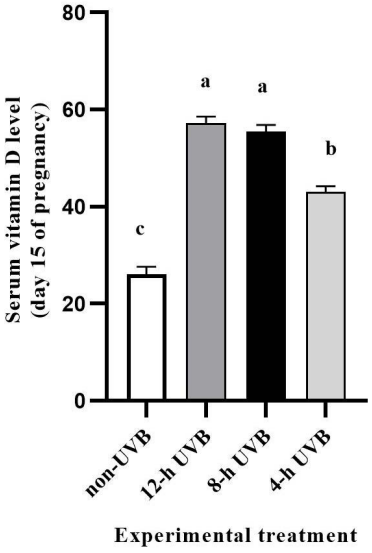

Figure 2: Effect of UVB radiation on serum 25 hydroxyvitamin D levels of does at day 15 of pregnancy.

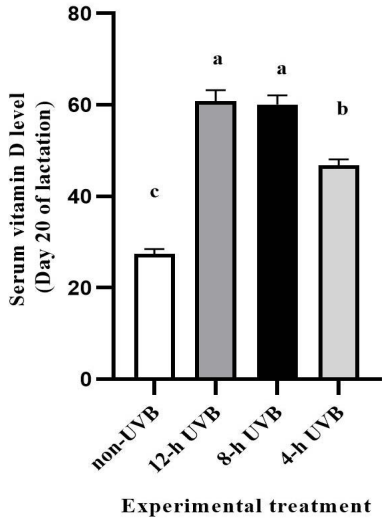

Figure 3: Effect of UVB radiation on serum 25 hydroxyvitamin D levels of does at day 20 of lactation.

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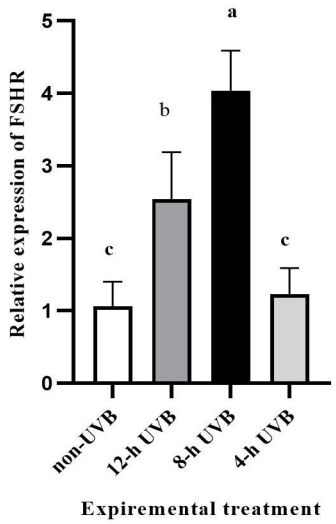

Figure 4: Effect of UVB radiation on FSHR relative gene expression in ovary.

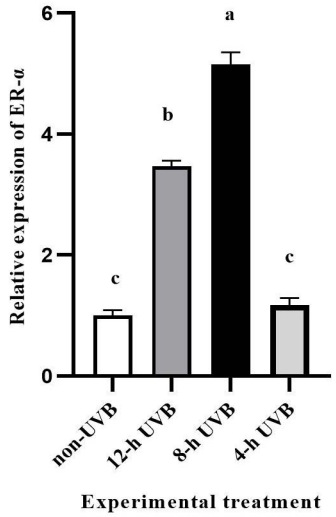

Figure 5: Effect of UVB radiation on ER-α relative gene expression in ovary.

CONCLUSIONS AND RECOMMENDATIONS

Environmental enrichment has been defined as any modification in the environment of captive animals that aims to enhance physiological and physical wellbeing (Newberry, 1995). Provision of environmental stimuli that could increase beneficial behaviour, decrease detrimental behaviour or do both could therefore be considered as a form of enrichment. The provision of ultraviolet light has been considered as a valuable form of environmental enrichment due to the fact that it enhances the quality of captive animals life and stimulates optimal physiological and psychological well-being (Shepherdson, 1998). The aim of this experiment was to examine how behaviour, reproductive and productive traits, serum levels of vitamin D and cortisol and levels of expression of reproductive genes in female rabbits are affected by exposure to various duration of UVB radiations.

The results show clear differences between rabbits experiencing different durations of UVB light in behaviour. Rabbits experienced 12-h of UVB light displayed higher levels of intake maintenance (feeding and drinking), general activity (stretching), non-intake maintenance (grooming) and exploratory behaviour compared to their relatives in non-UVB control group. These higher levels of feeding and drinking behaviours in the UVB-experienced rabbits could be a reflection to increased activity and improved colour vision in these animals. It has been shown that the supplementation of UVB lighting resulted in brighter lighting conditions and changed the spectral composition of the light (Smith et al., 2002), such a condition that might have enhanced active behaviours including feeding and drinking.

Physical activity is described as any movement resulted from muscle contraction leading to an increase in energy expenditure than during rest (Bjørgen, 2016). In the present study, rabbits exposed to UVB light showed higher levels of physical activity (stretching) and lower levels of inactivity (sitting and lying down) compared to their relatives in the non-UVB control group. These higher levels of activity and lower levels of inactivity could have been a reflection to the improved visual field and could therefore be considered an indicator of improved welfare in these animals. These results were not surprising, since there is a close association between exposure to UVB radiation and previtamin D3 synthesis in the skin (MacLaughlin et al., 1982).

Similar results of improvements in walking ability and engagements in more active natural behaviours after either exposure to UVB light or a dietary supplementation of vitamin D have been reported in chicken (Whitehead

et al., 2004). However, poorer walking ability was reported in broilers that received dietary rather than light supplementation of vitamin D3 (Ogbonna et al., 2022). Moreover, under the circumstances of the current experiment, and because rabbit does were housed singly and lacked social interactions, self-grooming was noted as a sole maintenance behavior. Therefore, the reduction in time spent lying down, resting and being inactive in rabbits experienced UVB light may be attributed to the increased time spent by these animals grooming, stretching, feeding and exploring the environment.

Rabbits subjected to UVB radiation in all provided durations were more explorative than those kept under non-UVB conventional lighting conditions. UV light has been shown to improve reflectance of substrate and to increase attractiveness of environmental objects to individual rabbits in terms of either hue or brightness (Hansen and Berthelsen, 2000). Therefore, this might explain the increased exploration levels displayed by rabbits kept in UVB visually-enriched environment.

Results of the current experiment have also shown that the expression of bar chewing behaviour was higher in rabbits kept in either non-UVB control or 4-h UVB group than rabbits in either 12-h or 8-h UVB group. This suggests that provision of short durations of UVB light (4 hours/day) was not sufficient to enrich the environment of rabbits and to reduce the incidence of abnormal bar chewing behaviour. The increased level of bar chewing behaviour in rabbits housed in conventional non-UVB lighting environment might have been appeared to compensate for the low time budget spent in other beneficial activity such as feeding, drinking, grooming and exploration. It might have also been appeared due to the lack of environmental stimulation and the impoverished housing conditions of standard lighting. Similar results of inability of low intensity UVB light (less than 10 W/m^2) amongst other materials environmental enrichments to reduce incidence of pecking in male turkey has been demonstrated (Lewis et al., 2000).

Although an extensive amount of research has been conducted to investigate the effects of UVB radiation on animals, very few of them have considered how UVB light supplementation affects reproductive performance traits particularly in female rabbits. The results of this experiment show that experiencing UVB light improved parameters of reproductive performance in female rabbits such as LWB, LWW, LWG from birth to weaning, mortality rate of kits and kindling rate of rabbits. LWB was higher in 12-h UVB group than in non-UVB control group, LWW and LWG from birth to weaning were higher in 8-h UVB group compared to non-UVB control group. Moreover, all durations of UVB light investigated in the current experiment reduced mortality rate of kits compared to those in non-UVB control group. This finding is not surprising and could be related to the increased synthesis of vitamin D in UVB-exposed animals. It has been suggested that vitamin D obtained as a result of exposure to UVB radiations is essential for hormonal functioning, embryogenesis, and organ development in human (Fernández et al., 2013). Previous reports illustrated that supplementation of vitamin D increased birth weight in infants (e.g. Harvey et al., 2014). On the other hand, other research reported no difference in the mean birth weight of infants despite confirming the role of vitamin D maternal supplementation in reducing the risk of low birth weight (LBW) (De-Regil et al., 2016).

The positive effect of maternal vitamin D supplementation either orally or through UVB exposure on birth size and risk of LBW might be mediated by changes in skeletal mineralization, fetal cell mass, function and metabolism (Kovacs, 2014). Therefore, the high mortality percentage recorded in kits of the non-UVB control group might be linked to the low birth weights and intrauterine growth restriction. Mortality rate in low-birth-weight neonates is 40 % higher than those with normal birth weight (Fanaroff and Walsh, 2011). An increase in progesterone production when granulosa cells were treated with 1,25(OH)2D in vitro has been previously reported (Merhi et al., 2014). An increase in production of estrogen, progesterone, estrone and insulin-like growth factor–binding protein1 has been reported in human ovarian cells incubated in vivo with 1,25-(OH)2D3 (Parikh et al., 2010). Therefore, the use of UVB radiation from natural or artificial sources as an approach of vitamin D3 synthesis should be considered.

Findings of the present study also demonstrated that baseline levels of serum 25-hydroxy vitamin D did not differ between experimental groups. However, the second and third blood samples that were collected at day 15 of pregnancy and day 20 of lactation, showed an effect of UVB light on the serum level of 25-hydroxy vitamin D. Does exposed to 12 and 8 hours of UVB light had higher serum 25-hydroxy vitamin D levels than those exposed to 4 hours of UVB light or those in the non-exposed control group. In human, vitamin D is considered deficient when the levels of 25-hydroxyvitamin D are less than 50 nmol/L or 20 ng/ml and toxic when 25-hydroxyvitamin D levels are over 374 nmol/L (Holick, 2007). Therefore, according to the level of serum 25-hydroxy vitamin D in pregnant and lactating does in control group (25.06±1.58, 27.40±1.12 nmol/L, respectively) and in 4-h UVB group (43.00 ± 1.22, 46.85 ± 2.30 nmol/L, respectively), the rabbits in these groups are considered vitamin D deficient, despite adequate levels of vitamin D3 were incorporated in standard commercial pelleted diet. While those in 12

(57.15 ± 1.41, 60.12 ± 2.35 nmol/L, respectively) and 8 hours UVB groups (55.40 ± 1.43, 60.12 ± 1.98 nmol/L, respectively) are not considered vitamin D deficient or likely to suffer vitamin D toxicity. It should be highlighted that rabbits in the current study were fed the same diet on a daily basis. Therefore, the possibility that food could be responsible for the significant difference in serum levels of 25-hydroxy vitamin D between groups can be ruled out. A similar increase in vitamin D levels in juvenile domestic rabbits following exposure to artificial UVB radiation for 12 hours daily has been previously reported (Emerson et al., 2014). Moreover, rabbits kept under free range conditions where they have a free access to sunlight had higher vitamin D levels than rabbits kept in hutches with no access to sunlight (Fairham and Harcourt, 1999). This suggests that exposure to UVB radiation either from sunlight or from artificial sources could efficiently increase circulating levels of 25-hydroxy vitamin D in serum.

The results of the current study showed that exposure of female rabbits to UVB radiation for 12 and 8 hours in particular resulted in up-regulations in mRNA level of *FSHR* and *ER-α* genes. The improvement in expression pattern of reproductive genes does not appear surprising. Vitamin D has been reported to play a role in gonads function including testis and ovary since VDR is expressed in these organs (Stumpf, 1995). Henceforth, uterine hypoplasia and impaired folliculogenesis were demonstrated in female VDR null mutant female mice (Yoshizawa et al., 1997). Moreover, vitamin D deficiency has been linked to gonadal insufficiency in rats and to a reduction by about 45% in number of sperms present in vaginal tract of females mated with vitamin D deficient male rats (Kwiecinksi et al., 1989). In addition, gonadotrophins including FSH and LH have been reported to have essential roles in steroid synthesis and ovarian follicular growth in human (Kakar et al., 1992). Likewise, follicular maturation and development were reported to depend on the successive actions of FSH and LH which is in turn mediated by *follicle stimulating hormone receptor gene* (*FSHR*) and *luteinizing hormone receptor gene* (*LHR*) (Ni *et al.*, 2007). Previous studies have indicated that there are specific *ER-α* and *FSHR* binding sites in the rabbit ovary (Lan et al., 2014; Han et al., 2014). As a result of receptor binding, the interactive action of FSH and LH promotes the production of ovarian steroid hormones (Ashley et al., 1999). Results concerning reproductive genes expression in rabbits ovary could be supported by a study in humans, where vitamin D has been linked to up-regulation of *FSHR* gene expression (Irani and Merhi, 2014). The up-regulation in the expressions of reproductive genes could also be clarified from a hormonal point of view, where vitamin D has been revealed to increase production of estrogen, estrone, progesterone and insulin like growth factor 1-binding protein 1 in human ovarian cells and to stimulate production of estrogen and progesterone in human placenta (Barrera et al., 2007). Additionally, other studies proved that vitamin D is capable of inducing changes in gene expression involved in follicular development and steroidogenesis in ovarian granulosa cells. They referred these findings to that treatment with vitamin D increased levels of aromatase tyrosine phosphorylation which in turn resulted in an increase in estradiol production (Merhi et al., 2014; Lee et al., 2014).

It could, therefore, be concluded that exposing female rabbits to either 12 or 8 hours of UVB radiation had the potential to not only increase behaviours that could reflect improved welfare but to also improve their reproductive and productive performance parameters, up-regulate reproductive genes expression and to elevate serum vitamin D levels to a safe range. However, from the economic and conservation of environment point of view, as experiencing 8 hours of UVB light mostly produced the same improvements in rabbits behaviour, health and reproductive profile of rabbits, and could potentially prolong the shelf life of UVB lamps, the inclusion of 8 hours of UVB radiation into the standard lighting cycle of rabbits could be recommended.

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NOVELTY STATEMENT

Our study is the first study that emphasizes the evaluation of the effects of different durations of UVB radiation (12-h UVB, 8-h UVB and 4-h UVB) on behaviour, reproductive performance indices and expression pattern of reproductive genes in female rabbits.

AUTHOR'S CONTRIBUTION

ASM designed the study protocol and supervised data collection procedures. UAA-I analyzed the data and shared in experimental protocol. MMF, RAD, AFA-E and AIA shared in writing the manuscript. All authors have finalized the experimental design and revised the manuscript and then contributed to, edited, and approved the final manuscript as submitted.

Conflict of interests

The authors have declared no conflict of interest.

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