Red LED light promotes hair follicle development to improve fibre quality in Su line Angora rabbit

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Abstract

Background: Light has crucial roles in animal physiological activities. This study aimed to investigate the effects of different colours of light-emitting diodes (LEDs) on rabbit fibre quality and hair follicle development. 50 three-month-old Su line Angora rabbits were randomly assigned to five groups. Treatment groups were exposed to same intensities of red, green and blue LED light under 16 h light:8 h dark photoperiod regimes. Control groups were exposed to white light and black. The trial spanned 73 days. Results: Results showed that LED colours exerted different effects on wool yield, fibre quality, hormones and hair follicle development. The wool yield of red group was higher than that of white, green and black groups (P<0.05). The shoulder fibre length of red group was higher than that of white and green groups (P<0.05). The coarse fibre diameter of white group was lower than that of green and black groups (P<0.05). The fibre diameter of red group was the lowest and was lower by 13.9% than that of control group (P>0.05). The coarse fibre ratio of green group was higher (13.31%) than that of red group (3.81%, P<0.05). The follicle groups of white, green and black groups consisted of 1 primary follicle associated with 3 or 4 secondary follicle groups and those of blue group consisted of 1 primary follicle associated with 5–10 secondary follicle groups. The follicle of red group consisted of numerous secondary follicles and a few primary follicles. In same magnification, the numbers of follicle groups of white, red, green, blue and black groups were 14.0, 16.5, 10.0, 11.67 and 11.0, respectively. The numbers of follicle groups of red and green groups significantly differed (P<0.05). Serum melatonin (MT) of red group was highest than that of white and green groups (P<0.01), higher than that of black group (P<0.05), serumTriiodothyronine (T 3) of red group was higher than that of white and black groups (P<0.05). Conclusions: Thus, the data reveal that red LED light can improve fibre quality, this may be due to red LED light which can enhance the secretion of melatonin to promote hair follicle development .

Background

Light, an environmental factor, markedly affects animal biological processes, such as seasonal reproductive cycles [1], pelage growth [2], spring moulting [3], appetite and weight changes [4] and horn growth [5]. Light plays an important role in animal production, and artificial lighting is often applied in livestock farms. Numerous studies on the effect of light on rabbits have been performed. Virag et al. [6] observed that milk production by rabbit does and kit litter weight under 1.5 light (L): 4 dark (D), 1.5L: 4D and 1L: 12D photoperiod regimes are higher than those under a 12L:12D photoperiod regime. Moreover, under shortened natural photoperiods, productivity is improved by 14 h of supplemental lighting per day using incandescent bulbs with 30 lux light intensity [7]. Mousa-Balabel [8] found that doe performance improves under daily fluorescent lighting with 20 lux light intensities and that doe performance under 14 h of lighting is better than that under 8, 10, 12, 14 or 16 h. However, Sun et al. [9] reported that the reproductive performance of rabbit does is unaffected by exposure to light with intensities of 60–100 lux.

The rabbit is a light-sensitive species. The feed intake of rabbit does is reduced under 12 h of daily intermittent lighting with 40 lux light intensity [6]. The European Food Safety Authority [10] stated that the light intensity of 50 lux is necessary for the visual illumination of conspecifics, investigation of

surroundings and stimulation of physical activity of rabbits. Light also regulates pelage growth [2]. However, few works have focused on the regulation of pelage growth by light, and most related works have focused on the use of low-level laser therapy (LLLT) to treat hair loss in humans. LLLT stimulates hair growth in individuals with male-pattern hair loss, female-pattern hair loss and alopecia areata with good outcomes and minimal side effects [11-13]. Fushimi et al. [14] reported that red LED light stimulates hair growth in mice and induces several potential mediators to stimulate hair growth from human dermal papilla cells. Fibre growth is also influenced by the characteristics of light (colour, length and intensity).

The present study aimed to determine the effects of different LED colours applied with the same photoperiod and intensity on the fibre quality and hair follicle development of Su line Angora rabbits. The results of this work provide a theoretical foundation for the use of LED light to improve wool production.

Methods

Animals, Diets and Feeding Procedures

Su line Angora rabbit is a new breed of rabbit from Jiangsu Academy of Agricultural Sciences and is raised as species resource in Liuhe animal science base. The owners of the Su line Angora rabbits permitted experimentation on their animals. Three-month-old Su line Angora rabbit wools were simultaneously sheared before the trial. A total of 50 rabbits (BW 2.245 ± 0.296 kg, similar wool yield) were housed in individual cages (66 cm × 44 cm × 52 cm) and provided with pellet feed ad libitum. The ingredients and chemical composition of the pellet feed are listed in Table 1. The basal diet was formulated in accordance with the recommended nutritional requirements for rabbits [15]. The feed was provided twice daily (08:30 and 16:00) in two equal portions. Animals were given free access to tap water throughout the experimental period. The body weight and feed intake of the rabbits were recorded before morning feeding every 14 days. After the trial, all rabbits continued to be raised as genetic resource in Liuhe animal science base.

Experimental design

The rabbits were randomly divided into five groups. Each group comprised 10 rabbits (5 males and 5 females), one cage of a rabbit is a replication. Rabbits in the experimental groups were exposed to red, green or blue LED lights (red, green, blue treatment groups) under a 16L: 8D photoperiod regime. Rabbits in the control groups were exposed to white light and black (control) under 16L: 8D and 0L:24D photoperiod regime. The trial spanned 73 days. An LED lamp band was obtained from the NVC Lighting Holding Limited and suspended at a distance of 50 cm from the rabbits. The light intensity was 40–50 lux.

Fibre sample and quality analysis

At the end of the trial, fibre samples were taken from the shoulders, backs and abdomens of the rabbits. The sampling areas had dimensions of approximately 2 cm × 2 cm. Fibre length was estimated using a

scaled ruler. A visual subjective test was conducted to identify fine and coarse fibres, and the dry weights of the fibres were determined to calculate coarse fibre ratio. Fibre diameter was measured with a random sample of 200 fibres and a projection microscope [16]. Moisture content was measured using an oven method [17].

Blood samples

At the end of the trial, blood samples were collected into test tubes through the ear vein at 9:00 in morning. Samples were collected before the morning feeding and watering. To isolate the serum, test tubes were first placed in a slanted position for 45 min at 4 °C and subsequently centrifuged for 15 min at 3,000×g. The serum was removed, and serum samples were frozen at −20 °C in 2 ml polyethylene tubes until analysis, which were used to test Melatonin (MT), Prolactin (PRL), Triiodothyronine (T_3), Thyroxine (T_4) and Growth hormone (GH).

Skin samples, staining and follicle analysis

At the end of the trial, skin samples were collected from the shoulders of the test rabbits. A pair of forceps was used to stretch a section of skin upwards. The skin section was excised by cutting parallel to the skin surface through connective tissue with a handheld scalpel blade. The skin samples were placed in plastic scintillation vials that contained 10% formalin buffered with sodium phosphate. The samples were then placed in small-mell individual baskets, dehydrated in a gradient ethanol series and cleared in Histoclear using Citadel tissue processor (Leica EG1150, Nussloch, Germany). The processed skin samples were embedded in paraffin using Leukhardt blocks. The embedded skin samples were sectioned in a transverse plane to the follicle line. Sections were prepared with thicknesses of 8 µm by using a base sledge microtome (Model Leica rm 213s, Nussloch, Germany). Approximately 60 sections were cut per sample, but only every other fifth section was retained. Twelve sections were retained per sample. All sections were deparaffinised and immersed in Histoclear for 2 min before staining and rehydrated in a gradient series of ethanol to water. Hematoxylin-eosin (HE) staining was performed to visualise the presence of follicular tissue compounds. Images of each section were acquired using Q-capture Pro 6.0. The number of follicle groups in one field of vision was counted under an inverted microscope and under the same magnification. The number of primary and secondary follicles in one follicle group was analysed. Primary and secondary follicles were identified on the basis of their associated gland structures.

Statistical Analysis

Data were analysed as a completely randomised block design through one-way ANOVA with SPSS 17.0 program. All cages of the Su line Angora rabbits served as the experimental units. Differences among means were tested using Duncan’s multiple range tests. Effects were considered significant at $P<0.05$.

Results

Effects of different LED colours on growth performance and fibre quality

The effects of LED colours on the growth performance and fibre quality of the Su line Angora rabbits are illustrated in Tables 2–5. Performance was influenced by different LED colours. Specifically, the wool yield of the red group was higher than that of the white, green and black groups ($P<0.05$). Final weight, average daily gain and feed intake were not influenced by LED colour ($P>0.05$). The shoulder fibre length of the red group was longer than that of the control and green groups ($P<0.05$). Back fibre length exhibited the same tendency as shoulder fibre length ($P>0.05$). Abdominal fibre length was unaffected ($P>0.05$). Fine and coarse fibre diameters were measured. Coarse fibre diameter was significantly influenced by different LED colours and was lower in the white group than in the green and black groups ($P<0.05$). However, fine fibre diameter was unaffected, and the fine fibre diameter of the red group was small and was lower by 13.9% than that of the white group ($P>0.05$). Coarse fibre ratio and moisture content are shown in Table 4. The coarse fibre ratio of the green group (13.31%) was higher than that of the red group (3.81%; $P<0.05$). The coarse fibre ratio of the other groups remained unaffected ($P>0.05$). Moisture content was unaffected by exposure to different LED colours ($P>0.05$). The serum T4 and GH were not influenced ($P<0.05$).

Effects of different LED colours on serum hormones

The effects of different LED colours on hair follicle development are shown in Table 6. The serum MT, PRL and T_3 were influenced by LED light. The serum MT of the red group was highest than that of the white and green groups ($P<0.01$), higher than that of black group ($P<0.05$). The serum PRL of the black group was lower than that of the white and green groups ($P<0.05$). The serumT_3 of the red group was higher than that of the white and black groups ($P<0.05$)

Effects of different LED colours on hair follicle development

The effects of different LED colours on hair follicle development are presented in Fig. 1 and Table 7. The hair follicle structures of groups exposed to different LED colours significantly differed. The follicle groups of the white, green and black groups consisted of 1 primary follicle associated with 3–4 secondary follicles. The follicle groups of the blue group consisted of 1 primary follicle associated with 5–10 secondary follicles. The follicle groups of the red group consisted of numerous secondary follicles and a few primary follicles. The follicle group numbers of the control, red, green, blue and black groups, were 14.0, 16.5, 10.0, 11.67 and 11.0, respectively. The follicle group numbers of the red and green groups significantly differed ($P<0.05$).

Discussion

Wool yield and fibre quality are important indicators of animal fur production. Fibre quantity and quality are influenced by numerous factors, including age, heredity, environment and nutrition. Considerable research has been conducted on the effects of heredity and nutrition on animal fur production. Different protein levels or supplements can influence the mohair production performance and fibre characteristics

of Angora goats [16, 18]. Zhang et al. [19] reported that the addition of 20 mg Cu/kg DM to basal diets (containing 5.60 mg Cu/kg DM) enhances the growth performance of cashmere goats. However, minimal research has been conducted on the effect of environmental factors, particularly lighting, on fur production. The present study aimed to investigate the effects of different LED colours on fibre quality. Results showed that wool yield and fibre characteristics are influenced by different colours of LED lighting applied with 16L: 8D photoperiod regimes. Red LED light can increase wool yield and fibre length and decrease fine fibre diameter and coarse fibre ratio. Sheen et al. [20] found that in mice, anagen entry is faster under red light than under green and blue lights. Han et al. [21] reported that in an in vitro culture model, 655 nm red light + LED promotes human hair growth. Some reports have shown that fibre growth is regulated by melatonin. In this trial, we also tested the serum hormones including melatonin and found Red LED light could enhance the concentration of serum melatonin and T_3. In animals, light with a certain level of intensity sends a signal to the pineal gland to initiate or terminate melatonin synthesis and secretion, and melatonin transmits a signal with a circannual rhythm to regulate seasonal reproduction and other biological processes, such as hibernation, migration and pelage changes [22]. Numerous studies have been conducted on melatonin. Coelho et al. [23] reported that in the southern hemisphere, hair and wool ewe lambs exhibit the same annual pattern of plasma melatonin concentration under natural photoperiods at low latitudes. Exogenous melatonin treatment during spring positively affects the medium- and long-term indices of the wool quality of Rasa Aragonesa ewes [24]. Moreover, exogenous melatonin can improve wool production and fibre quality [25]. Duan et al. [26] found that melatonin implantation (2 mg/kg BW) on two occasions (late April and June) increases cashmere yield by inducing cashmere fleece growth and decreases fibre diameter without changing dam growth rate or reproductive performance. Yang et al. [27] reveal that melatonin serves to promote secondary hair follicle development in early postnatal cashmere goats and expands our understanding of melatonin application in cashmere production. Cong et al. [28] stated that melatonin implantation during the winter solstice can effectively extend the cashmere growth phase of Liaoning cashmere goats. Melatonin treatment led to an increase in both the quantity and quality of cashmere fibre. Therefore, the improvement in fibre quality through light treatment may be related to melatonin. In the present study, the effects of red LED light are better than those of natural light or other LED light colours. Therefore, red LED light plays a key role in the fibre growth of Angora rabbits.

To validate the abovementioned outcome, the development and structure of hair follicles were further determined and observed. Results showed that the red group has a follicle group number of 16.5 and exhibits follicle groups that comprise numerous secondary follicles and a few primary follicles. This result agrees with the result for fibre quality under red LED light. Few reports have focused on the changes in the characteristics of hair follicles under light treatment. Lanszki et al. [25] observed that melatonin treatment increases the number of active follicles (lateral primaries and secondaries) per hair follicle group by 32%. Melatonin is the main factor that affects follicle activities, and light can influence melatonin synthesis and secretion. Red LED light may increase the number of secondary follicles in rabbits or other fur animals (e.g. sheep, cashmere goats) by changing melatonin levels. Nevertheless, the

relationship between different colours of LED light and melatonin warrants further study to elucidate the mechanism that underlies the influence of LED light on fibre quality.

Conclusions

Wool yield, fibre quality (length, diameter and coarse fibre ratio) and hair follicle structure are affected by red LED light. Red LED light can enhance the concentrations of serum melatonin and T_3, promote the development of secondary follicles. Therefore, red LED light may promote hair follicle development through melatonin, and further improve fibre growth and quality. Also further studies are required to identify the mechanism that underlies the influence of LED light on fibre quality.

Abbreviations

LED: light-emitting diode h: hour L: Light D: Dark LLLT : low-level laser therapy MT: Melatonin PRL: Prolactin T_3: Triiodothyronine

T4: Thyroxine GH: Growth hormone HE: Hematoxylin-eosin

Declarations

Ethics approval and consent to participate

The experiment was approved by the Research Committee of the Jiangsu Academy of Agricultural Sciences and was conducted in accordance with the Regulations for the Administration of Affairs Concerning Experimental Animals (Decree No. 2 of the State Science and Technology Commission on November 14, 1988).

Availability of data and material

All data generated or analysed during this study are included in this manuscript.

Consent for publication

Not applicable.

Competing interests

We declare that we do not have any commercial or associative interest that represents a conflict of interest in connection with the submitted work. The authors alone are responsible for the content and writing of this article.

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Authors' contributions

FQ conceived of the study and drafted the manuscript, FQ and JW carried out the experiments. LS, XP, CZ, XZ, JL and SL assisted with the sample collection and analysis. JY and PZ participated in the study's design and coordination. All authors read and approved the final manuscript.

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Tables

Table 1 Ingredients and chemical composition of the basal diet used in this work

Item	
Ingredient	%[1]
Alfalfa powder [2]	38.0
Corn	26.0
Wheat bran	13.0
Soybean meal	13.2
Yeast powder	2.0
soybean oil	2.5
Salt	0.3
Premix[3]	5.0
Chemical composition	
Digestible energy (MJ/kg)[4]	10.15
DM	89.47
Crude protein	16.46
Crude Fibre	13.85
Calcium	1.21
Phosphorus	0.74
Lysine	0.93
Methionine +Cysteine	0.86

[1]As fed basis.

[2]Content per kg of Alfalfa Powder: 90.47% DM, 6.11 MJ/kg DE, 16.02% CP and 27.35% CF.

[3]Content per kg of premix: 5320 mg of $FeSO_4.H_2O$, 1080 mg of $CuSO_4.5H_2O$, 560 mg of $MnSO_4.H_2O$, 3652 mg of $ZnSO_4.H_2O$, 1000 mg of $CoCl_2.6H_2O$, 180,000 IU of vitamin A, 18,000 IU of vitamin D and 900,000 IU of vitamin E.

[4]All values represent the measured values except for DE. DE was calculated by using known DE values for basal diet ingredients.

Table 2 Effects of different colours of LED lights on the growth performances of Su line Angora rabbits

Item	White	Green	Red	Blue	Black	SEM	P-value
Initial weight (g)	2252.2	2240.8	2251.2	2241.3	2241.5	54.1	1.000
Final weight (g)	3239.3	3353.0	3310.7	3283.7	3337.0	45.7	0.951
ADG (g/day)	13.52	15.23	14.51	14.27	15.01	0.30	0.429
Feed Intake (g/day)	150.3	155.7	153.5	152.8	157.2	2.25	0.910
Wool yield (g)	106.8^{b}	106.3^{b}	121.1^{a}	110.2^{ab}	106.0^{b}	1.95	0.050

White = White light group; Green = Green LED light group; Red = Red LED light group; Blue = Blue LED light group; Black = Black group

a,b Values within a row with different superscripts differ significantly at $P<0.05$.

Table 3 Effects of different colours of LED lights on the fibre lengths of Su line Angora rabbits

Item	White	Green	Red	Blue	Black	SEM	P-value
Shoulder fibre length (mm)	42.7^{b}	42.7^{b}	57.8^{a}	48.5^{ab}	44.5^{ab}	2.04	0.038
Back fibre length (mm)	49.0	47.8	54.5	50.0	46.5	1.97	0.761
Abdominal fibre length (mm)	45.1	44.4	40.1	38.3	34.3	2.65	0.777

White = White light group; Green = Green LED light group; Red = Red LED light group; Blue = Blue LED light group; Black = Black group

[a,b] Values within a row with different superscripts differ significantly at $P<0.05$.

Table 4 Effects of different colours of LED lights on the fibre diameters of Su line Angora rabbits

Item	White	Green	Red	Blue	Black	SEM	*P-Value*
Diameter of fine fibre (μm)	14.14	13.56	12.18	12.84	12.36	0.34	0.344
Diameter of coarse fibre (μm)	28.7^{b}	43.9^{a}	38.2^{ab}	35.9^{ab}	41.0^{a}	1.76	0.033

White = White light group; Green = Green LED light group; Red = Red LED light group; Blue = Blue LED light group; Black = Black group.

^{a,b} Values within a row with different superscripts differ significantly at $P<0.05$.

Table 5 Effect of different colours of LED lights on moisture contents and coarse fibre ratios of Su line Angora rabbits

Item	White	Green	Red	Blue	Black	SEM	P-value
Moisture content (%)	14.21	14.00	13.73	13.06	13.48	0.29	0.797
Ratio of coarse fibre (%)	12.06^{a}	13.31^{a}	3.82^{b}	5.30^{b}	12.07^{a}	1.27	0.014

White = White light group; Green = Green LED light group; Red = Red LED light group; Blue = Blue LED light group; Black = Black group

^{a,b} Values within a row with different superscripts differ significantly at $P<0.05$.

Table 6 Effect of different colours of LED lights on serum hormones of Su line Angora rabbits

Item	White	Green	Red	Blue	Black	SEM	P-value
MT (ng/L)	120.6^{c}	129.2^{bc}	151.6^{a}	141.3^{ab}	130.2^{bc}	2.71	0.001
PRL (ng/mL)	70.7^{a}	69.9^{a}	65.7^{ab}	60.6^{ab}	56.0^{b}	1.84	0.037
T_3 (ng/mL)	1.56^{b}	2.04^{ab}	2.16^{a}	2.01^{ab}	1.46^{b}	0.09	0.048
T_4 (ng/mL)	63.6	60.7	64.9	64.1	60.9	1.14	0.714
GH (ng/mL)	2.05	1.99	1.74	1.73	1.86	0.06	0.409

White = White light group; Green = Green LED light group; Red = Red LED light group; Blue = Blue LED light group; Black = Black group;

a,b or b,c Values within a row with different superscripts differ significantly at $P<0.05$, a,c Values within a row with different superscripts differ significantly at $P<0.01$.

Table 7 Effect of different colours of LED lights on skin hair follicles of Su line Angora rabbits.

Item	White	Green	Red	Blue	Black	SEM	P-value
Primary follicle:secondary follicle[1]	1: 3–4	1: 3–4	few: many	1: 5–10	1: 3–4	-	-
Follicle group number	14.00[ab]	10.00[b]	16.50[a]	11.67[ab]	11.00[b]	0.78	0.027

White = White light group; Green = Green LED light group; Red = Red LED light group; Blue = Blue LED light group; Black = Black group

[1]Primary and secondary follicles in one follicle group.

[a,b] Values within a row with different superscripts differ significantly at $P<0.05$.

Figures

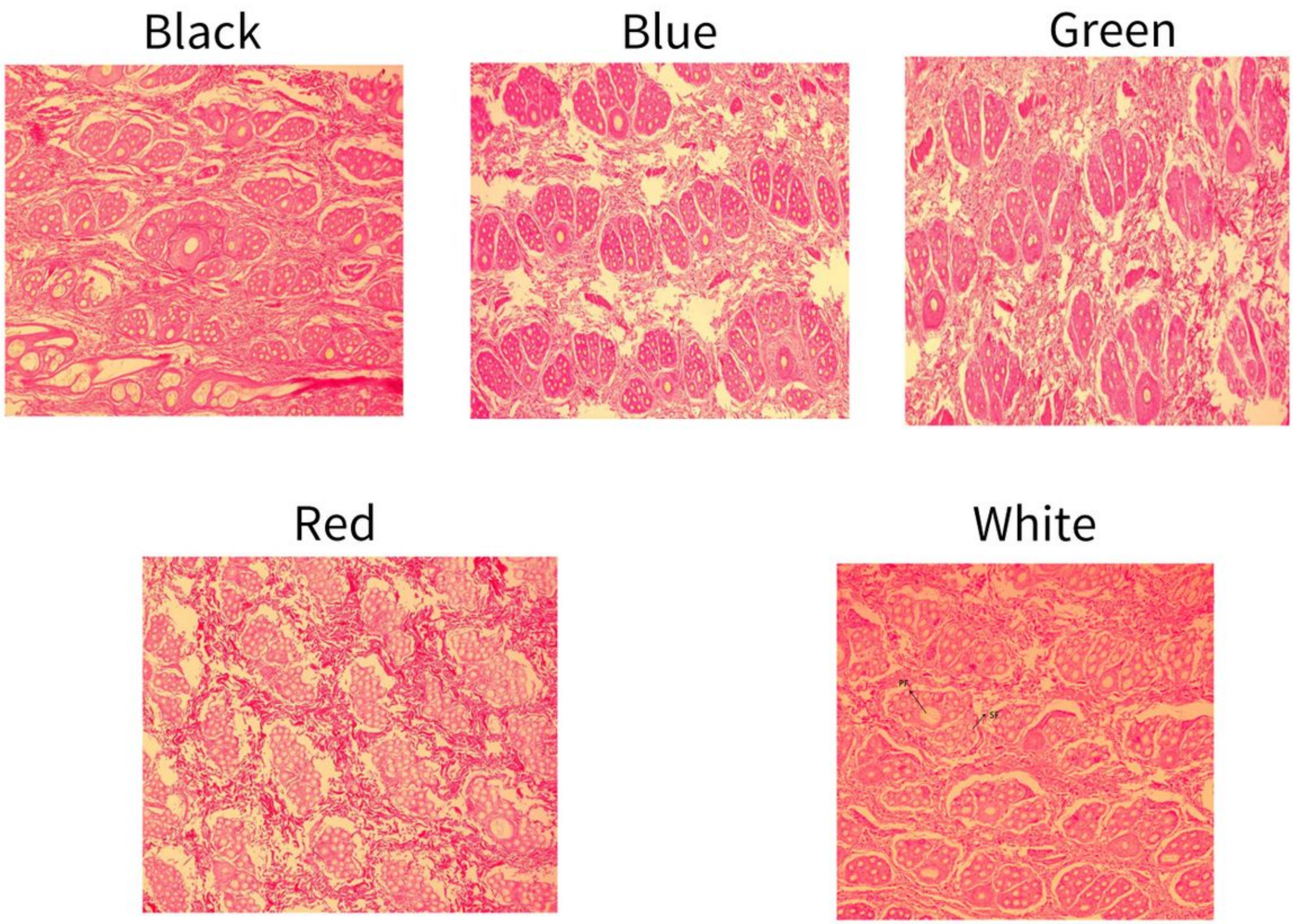

Figure 1

White = White light group; Green = Green LED light group; Red = Red LED light group; Blue = Blue LED light group; Black = Black group. Skin tissue sections with hair follicles were observed under 100×

magnification (n = 5). PF and SF represent primary follicle and secondary follicle, respectively. The number of PFs and SFs in one follicle group was analysed, and the number of follicle groups in each section was counted under the same magnification. In figures, the follicle groups of the white, green and black groups consisted of 1 primary follicle associated with 3–4 secondary follicles, the follicle groups of the blue group consisted of 1 primary follicle associated with 5–10 secondary follicles, the follicle groups of the red group consisted of numerous secondary follicles and a few primary follicles. The follicle group numbers of the control, red, green, blue and black groups, were 14.0, 16.5, 10.0, 11.67 and 11.0, respectively.

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