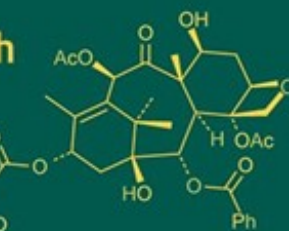
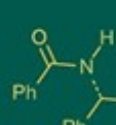


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Effects of blue and red LED lighting on circadian rhythm, immune function, physiological responses, and stress hormones (cortisol and norepinephrine) in sheep

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ABSTRACT

Abstract: In intensive and semi-intensive sheep production, artificial lighting is becoming an increasingly crucial management weapon, although the physiological effects of particular wavelengths of light in sheep are not well understood. Multiple monochromatic narrow-band lights, such as blue (around 450-490 nm) and red (around 620-700 nm), are now available, which can deliver intense light at specific wavelengths - something previously not possible with incandescent and fluorescent sources of light. The mammalian retina also contains intrinsically photosensitive Retinal Ganglion Cells (ipRGCs) with high sensitivity to short-wavelength (blue) light, 57, which have important implications downstream beyond vision. These are efferent, or non-visual pathways, which connect the retina with the Suprachiasmatic Nucleus (SCN), and with the pineal gland and the Hypothalamic-Pituitary-Adrenal (HPA) and Sympathetic-Adrenomedullary (SAM) pathways that influence circadian rhythm, the Pineal gland, immune competence, and the release of stress hormones (cortisol, norepinephrine). This review summarizes the evidence for sheep and, if applicable, from closely related ruminant models as well as avian and mammalian models of the physiological effects of blue lights versus red lights. Review also includes physiological aspects like body temperature, heart rate, respiratory rate, and feed consumption in regard to lighting in the barn, handling and transport, and animal welfare, and concludes with practical implications. The gaps in knowledge around sheep are identified and priority areas for controlled research proposed.

Keywords: Sheep, LED lighting, Blue light, Red light, Circadian rhythm, Melatonin, Immune function, Cortisol, Norepinephrine, Animal welfare

1. Introduction

One of the strongest zeitgebers (environmental factors) regulating mammalian physiology is light. In addition to its role in vision, light synchronizes the endogenous clock, controls the nocturnal secretion of pineal melatonin, and via linked neuroendocrine pathways affects immune competence, thermoregulation, cardiovascular function and the activity of the two major stress axes: hypothalamic-pituitary-adrenal (HPA) axis and the Sympathetic-Adrenomedullary (SAM) axis which finally activate the release of cortisol or the catecholamines epinephrine and norepinephrine, respectively (Navara *et al.*, 2007) [1]. Sheep (*Ovis aries*) are highly photoperiodic and the effects of controlled lighting and exogenous melatonin on their reproductive seasonality have been known for many years. Sheep have a known sensitivity to light duration (photoperiod), and thus should also be sensitive to the quality of light, i.e. the spectrum or colour of the lights used. There has been comparatively little direct work on this aspect of sheep sensitivity. In the past, lighting in the farming sector has been based on incandescent light sources and fluorescent tubes, which produce relatively disorganized, broad-spectrum light. This view has now changed significantly with the use of Light Emitting Diode (LED) technology for livestock dwellings: LEDs can be made to emit such a narrow spectrum of light that it is monochromatic at almost any possible wavelength, they are energy efficient, durable and rapidly becoming inexpensive, and they allow a spatial and energetic control of the photoperiod and the spectral choice for periods that is both more precise and reproducible (Horodincu *et al.*, 2023) [2].

Two wavebands have been especially researched in different livestock species: one waveband of blue light around 450-490 nm and one waveband of red light around 620-700 nm. These are two extreme colors in visible light spectrum, contrasting not only by their photon energy but also in their ability to activate melanopsin containing ipRGCs, as well as in their ability to penetrate the biological tissue (Galan *et al.*, 2025) [3]. Comparative studies in poultry, rodents, and humans suggest that blue light is the strongest suppressive cue to nocturnal melatonin and strongest stimulus to phase shifts of the circadian system of the clock, while red lights are relatively 'circadian-neutral' and allow for close to normal nocturnal melatonin output yet support vision under low ambient light. These spectral differences also manifest in identifiable

differences in immune parameters, physiological indicators and levels of stress hormones in a number of farmed species, and there is an increasing interest in the possibility of applying the same to enhance welfare and productivity in sheep farming, transport, and lambing management (Hofmann *et al.*, 2020) [4].

2. Light perception and non-visual photobiology in sheep

Like in other mammals, the retina of sheep possesses classical rod and cone photoreceptors important for image forming or vision function, and a small, but highly relevant population of intrinsically photosensitive retinal ganglion cells (ipRGCs). The ipRGCs contain the photopigment melanopsin for which a peak spectral sensitivity is usually situated in the blue part of the visible spectrum (around 460–480 nm). In contrast to the rods and cones, ipRGCs are not primarily involved in the picture of conscious pattern vision; their axons connect to the mammalian's master circadian pacemaker, the Suprachiasmatic Nucleus (SCN) of the hypothalamus in the direct Retinohypothalamic Tract (RHT). As melanopsin is most sensitive to the blue (short-wavelength) photons, their effect is more than is expected for red light of similar photon flux or illuminance, in terms of RHT signalling to the SCN. Once activated, the SCN sends timing signals along multisynaptic pathways to the paraventricular nucleus of the hypothalamus which in turn sends signals to the superior cervical ganglion and from there to the pineal gland, where they influence the nocturnal synthesis of melatonin from serotonin using the enzymes arylalkylamine *N*-acetyltransferase (AANAT) and hydroxyindole-*O*-methyltransferase (HIOMT/ASMT) (Wyse *et al.*, 2018) [5].

Apart from entraining circadian rhythms, ipRGC-SCN-pineal circuits also communicate extensively with both the Paraventricular Nucleus (PVN) which is the source of Corticotropin-Releasing Hormone (CRH) neurons activated when there are pro-HPA axis activation signals, and pre-autonomic neurons that control the sympathetic pathway to the adrenal medulla. This anatomical connection is where it likely comes from mechanistically that light colour is seen to affect not just melatonin and circadian phase, as demonstrated in Sections 5 and 6, but on the activity of the two major stress hormone axes. In mammals, including sheep, the eye is the most important (and possibly only) sensory pathway for the transmission of light information to the central circadian and neuroendocrine systems, and the eye is also a site of well-developed peripheral, extra-ocular photoreception in some contexts. In birds, deep-brain photoreceptors have been described as well (Song *et al.*, 2025) [6]. It is also important to note that the positioning of the light fixture in relation to the height of the sheep when they are grazing will affect light irradiation reaching each eye, due to the mixing of the different wavelengths within the pupil, the vocal pigmentation of the eye, and the size and conformation of wool around the head; therefore, even though the nominal output of a fixture is low, the actual light irradiance received by each eye of the sheep might not be, and is likely to be less than, this (Hayashi *et al.*, 1985) [7].

3. Circadian rhythm and melatonin regulation

Melatonin secretion, body temperature, locomotor activity and feeding behaviour exhibit strong diurnal and annual rhythms in sheep and melatonin is probably the main endogenous signal that reflects and regulates annual reproductive cycles. A peak in plasma melatonin levels is observed shortly after the onset of darkness and is maintained high throughout the night, and decreases quickly around dawn when light levels are high. The profile is very light-sensitive, e.g. short light pulses during nighttime periodize the oscillator and prevent melatonin production, which is a situation extensively utilised in sheep and goat reproductive control, with long-day light to induce an advanced profile, or by using melatonin implants to achieve a delayed profile (Kyriazopoulou, 2021) [8]. The effect of exposure to the blue light

sources and to the red sources at matched illuminance level on the suppression of melatonin levels appears most severe in humans and rodents exposed to blue light in the late evening, whereas red light at matched illuminance level allows a significantly larger recovery from melatonin levels during the night.

Results in poultry broadly agree: that monochromatic blue light has been linked to increased circulating melatonin for a select few lighting-combination conditions when combined with green light, suggesting a more complex interaction between the spectral colour of light and melatonin output is needed that is influenced by timing, intensity, developmental stage and photophase/scotophase or scotophase light delivery (Cortes-Mercado, 2026) [9]. More recent studies on rodents have demonstrated that the photophase (daytime) administration of LED light (either blue, green or red) affects pineal indole metabolism metabolites such as *N*-acetylserotonin and melatonin in animals even when they are only exposed to light during the day, suggesting that the effect of wavelength does not only occur at night (Zhang *et al.*, 2022) [10]. The major physical and physiological differences between blue and red LED light are summarised in Table 1 to understand their significance for circadian and neuroendocrine outcomes.

4. Immune function responses to blue and red light

Melatonin is also not just a circadian Zeitgeber: It affects the proliferation of lymphocytes, antibody production and the balance of cytokines via membrane-bound (Mella/Mellb/Mellc; also called MT1/MT2) and nuclear (ROR α /ROR β /ROR γ) receptors expressed on immune cells and lymphoid tissue. Given that cortisol is generally immunosuppressive, and that light affects immune competence through stress-hormone-dependent pathways, too, light colour can affect immune competence, either through the melatonin pathway, or via stress-hormone pathway, with actuators of the latter including sympathetic and hypothalamic-pituitary-adrenal pathways, made up of affiliated regions (Russell *et al.*, 2015) [11]. Since cortisol is generally immunosuppressive, light colour may affect immune competence both via the melatonin pathway and via the stress-hormone pathway, which includes sympathetic pathway and hypothalamic-pituitary-adrenal pathway, with accompanying regions. Some of the best mechanistic data exists for a monogastric model, in chickens. The red light alone reared chicks exhibited aggravated oxidative stress and inhibited lymphocyte proliferation, while reared in combination with the blue light (especially the green-to-blue lighting sequence) significantly improved plasma melatonin levels, diminished oxidative stress markers and promoted lymphocyte proliferation, follicular area and antibody titre in the bursa of Fabricius, which were partially reversed upon the pharmacological blocking of melatonin receptor signalling by lights alone (Mendez *et al.*, 2024) [12]. The study shows a direct receptor-mediated mechanism between light-induced melatonin secretion and humoral immune output in a produced species, and highlights that light colour effects on immunity are not fortuitous but its operation follows receptor-mediated “neuroendocrine-immune” signaling pathway(s).

In total, in dairy cows, the light-wavelength group (compared to red light or darkness) that he or she was exposed to showed measurable increase in antioxidant capacity, immune indices and reproductive performance; although this trend was only recently demonstrated in dairy cows with yellow light, it also has been confirmed with other wavelengths in other ruminant species (light-wavelength dairy cow study, 2024/2025). There is a reasonable physiological basis for believing that appropriately timed exposure to blue-light (similarly as during the daytime photophase) could similarly benefit immune parameters in sheep as has been demonstrated with several of the other shown wavelengths above, where the beneficial effects appear to be mediated by melatonin pathways, especially in ruminants and poultry where direct evidence to support this is available (Mendez *et al.*, 2016) [13].

5. Physiological responses to blue and red LED lighting

In addition to circadian and immune measures, color of lights has also been correlated to various other general physiological parameters in livestock, such as core and peripheral temperature, heart rate, respiration rate, intake of feed and water and locomotor activity. The parameters are relevant to sheep farming practice in that they are easily measured on-farm and are widely used as composite parameters with cortisol concentration and concentrations of catecholamines to indicate an animal's physiological state and its welfare status (Kalafatakis

et al., 2019) [14]. Benefits found in a blue-enriched (or blue-shifted) light rearing environment for birds and pigs have been linked to the lower level of light-related arousal and increased calmness that has been reported in incubators and conventional facility lighting, as well as reduced levels of activity, and, in some experiments, improved feed conversion ratio in some species with bimodal melatonin release patterns. Some poultry and pig farms have been keen to adopt red light to suit husbandry activities that only need to be done at night that it would either not cause any disruption to the circadian rhythm or melatonin levels of birds, or would have a less dramatic effect on them when they are resting or sleeping (Stehle *et al.*, 2011) [15].

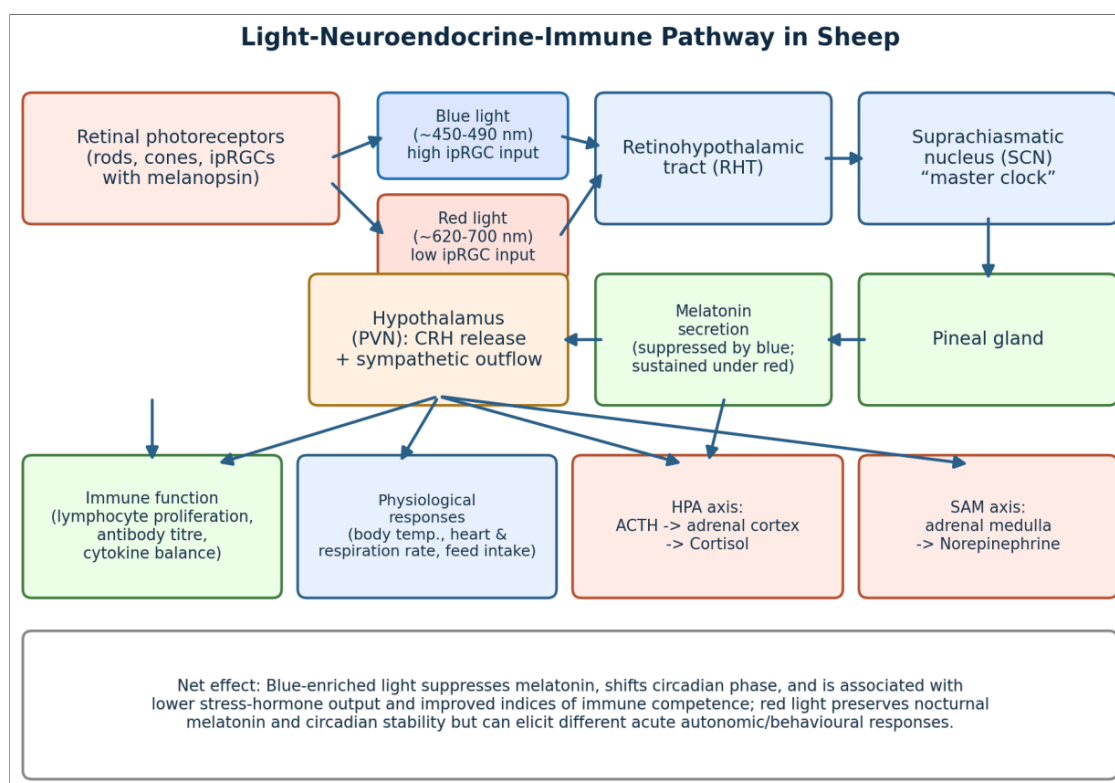


Figure 1. Schematic overview of the light-neuroendocrine-immune pathway relevant to sheep

Although physiological responses of sheep to monochromatic light colour are limited, the general conclusions from the sheep and goat photoperiod literature are that adjustment of metabolic rate, feeding pattern and thermoregulatory set points follow the season are reliably modified via light manipulation and these pheromone effects result from action on the hypothalamus and peripheral organs, primarily through melatonin mediated pathways (Everds *et al.*, 2013) [16]. The evidence for spectral lighting can be extrapolated to suggest that a combination of a daylight blue regime (to promote alertness, feeding and health-associated phenomenon) with red or low intensity lighting during transmeridian periods (to maintain melatonin production and reduce disturbance during the night) may be optimum for both productivity and welfare for housed sheep, though this regime has not yet been carefully tested in sheep.

6. Stress hormones: cortisol and norepinephrine

Cortisol is the main glucocorticoid product of the HPA axis; and norepinephrine a primary catecholamine effector of the SAM axis, are two widely used physiological markers of acute and short-term stress in livestock studies. Convergent connections from the SCN and light-responsive retinal pathways insure a direct pathway to the hypothalamus Paraventricular Nucleus (PVN) by which light colour, intensity

and timing can independently affect stress-hormone output, independent of any overtly stressful handling event. The greatest experimental evidence that light colour itself affects cortisol has come from a controlled, human circadian study, where both narrow-band blue and red light exposures proved to either increase or decrease cortisol levels, compared to a dark control, while only blue light suppressed melatonin levels at night (Figueiro & Rea, 2010). Importantly, this dissociation suggests the nonvisual retinal pathway that regulates melatonin suppression is different from the pathway(s) that regulates cortisol responses to light and both combinations of blue and red light can serve as drivers of the circadian system and neuroendocrine function, but may do so to different extents and end in different fates (Kiehn *et al.*, 2017) [17].

On a practical level, this research may permit some of the chances of HPA activation at night to be reduced through the use of a combination of blue enriched light in the day, dim/red light in the scotophase, and of course during normal night checks, to lessen the unnecessary cortisol elevation that might occur with lighting-induced circadian disruption in the housed sheep. The direct evidence for this particular claim is not available in sheep, comes from related species and extrapolated from human and rodent circadian-endocrine studies so is listed here as a possible although not established finding, and as a recommended topic for confirming studies in sheep in Section 8.

Table 1. Comparative characteristics of blue and red LED lighting relevant to circadian and neuroendocrine outcomes

Characteristic	Blue LED light (~450-490 nm)	Red LED light (~620-700 nm)
Photon energy	Higher (shorter wavelength)	Lower (longer wavelength)
Melanopsin/ipRGC stimulation	Strong; near peak sensitivity	Weak; well below peak sensitivity
Nocturnal melatonin suppression	Pronounced and rapid	Minimal to moderate; melatonin largely preserved
Circadian phase-shifting potency	High	Low to moderate
Tissue/ocular penetration	Lower penetration; more scattering	Greater penetration; less scattering
Typical behavioural effect	Associated with heightened alertness, reduced resting behaviour if used at night	Associated with calmer behaviour; used for low-disruption night visits
Common livestock application	Daytime supplementation, immune/growth modulation, extending photoperiod	Night-time low-disruption lighting, minimal melatonin interference

7. Comparative synthesis across species and implications for sheep

Comparative studies of different types of monochromatic blue versus red LED lighting are scarce in sheep, and so the evidence base for this review has been a mix of humans, rodents, poultry, and cattle. Despite this wide diversity in literature, one important pattern can be discerned: blue light with similar or comparable radiant intensity elicits more potent effects on melanopsin-mediated pathways, including melatonin suppression and circadian phase-shifting compared to red

light, and red light has relatively greater efficacy in the protection of melatonin suppression and circadian phase stability. Beyond this common origin, species vary in the specific physiological / immunological effects seen, depending on differences in retinal architecture, activity rhythm (diurnal / crepuscular / nocturnal) or housing situation or effect measured. To help in extrapolating results to sheep-specific uses, but also to make it clear which results are of ovine origin and which of related species, representative findings from the comparative literature covered in this review are summarised by species, light treatment and principal findings in [table 3 \(Andronachi *et al.*, 2025\) \[18\]](#).

Table 2. Summary of physiological and immune parameters reported under blue versus red light exposure across species

Physiological/immune parameter	Reported association with blue light	Reported association with red light	Primary evidence base
Nocturnal melatonin	Strongly suppressed	Largely preserved / near-normal rhythm	Human, rodent, ruminant studies
Circadian phase shift	Strong phase-shifting effect	Weak phase-shifting effect	Human, rodent studies
Lymphocyte proliferation / antibody titre	Enhanced (in combination lighting regimes, melatonin-dependent)	Reduced under continuous exposure in some poultry trials	Poultry (bursa/B-lymphocyte) studies
Oxidative stress markers	Generally reduced when paired with elevated melatonin	Elevated under prolonged single-colour exposure in some avian trials	Poultry studies
Resting behaviour / activity level	Calmer resting behaviour reported in some livestock trials	Used preferentially for low-disruption night handling	Poultry, swine husbandry practice
Heart rate / autonomic activation	Context-dependent; can rise with nocturnal exposure via arousal	Context-dependent; generally lower disruption at night	Human autonomic studies; livestock handling reports
Cortisol (acute)	Both wavelengths can acutely alter cortisol; pattern time-of-day dependent	Both wavelengths can acutely alter cortisol; pattern time-of-day dependent	Human circadian-cortisol study (Figueiro & Rea, 2010)

This synthesis lends credence to several extrapolations and it needs to be confirmed outright for others. Sheep have a similar core melanopsin-RHT-SCN-pineal architecture to other ruminants, such as humans or rodents, and are also a known very sensitive species to photoperiod and light-pulse effects on melatonin, making it reasonable to assume that blue LED light would be more effective than red LED light of the same intensity in causing suppression of nocturnal melatonin and altering circadian phase in sheep ([Mezaal *et al.*, 2025\) \[19\]](#). Second, from the existing literature on the immune benefits of blue-inclusive lighting regimes in poultry, plus the immune and antioxidant benefits shown with different wavelengths in dairy cattle, there is a logical case to be made for expecting similar immune bene-

fits with appropriately-timed blue-light exposure in sheep. Third, the human results demonstrate a sizeable effect of both blue and red light on cortisol, which appears to occur through pathways at least partially independent to melatonin suppression; thus, to extrapolate results of cortisol findings in sheep from measures of melatonin suppression alone, additional measurements of cortisol under both lighting colours would be required ([Astuti, 2025\) \[20\]](#). Fourth, repeated observations of the less consistent latrines of NE responses compared to cortisol responses to common physical and psychological stressors should not warrant using only norepinephrine measures in future studies of light-colour effects on the SAM axis, as some measures of cortisol, heart rate and behavior should be utilized as well.

Table 3. Representative comparative findings on blue versus red (or related) light treatments across species

Species / model	Light treatment compared	Key reported outcome
Human (controlled circadian protocol) Human (healthy adults) Chicken (broiler chicks)	Blue (~480 nm) vs red (~625 nm) LED, 40 lux, nocturnal	Only blue light suppressed nocturnal melatonin; both blue and red altered cortisol
	Blue (464 nm) vs red (631 nm) LED, evening exposure	Blue light produced stronger, more sustained melatonin suppression after ~2 h
	Red, green, blue single and combined monochromatic light	Red light exacerbated oxidative stress; green-to-blue sequence raised melatonin and enhanced B-lymphocyte proliferation and antibody titre
Rat (Wistar, photophase exposure) Dairy cattle	Blue, green, red monochromatic LED during daytime	Daytime light colour altered diurnal pineal indole (melatonin pathway) rhythms
	Natural dark vs red vs yellow nocturnal light	Yellow light raised endogenous melatonin and improved immune/antioxidant/reproductive indices relative to red light and darkness
Sheep (various procedures) Goat (transport)	Isolation, restraint, transport simulation (not spectral colour)	Cortisol consistently rose with stressors; norepinephrine responses were comparatively modest or non-significant
	Road transport (not spectral colour)	Epinephrine rose sharply and returned to baseline; cortisol peaked within 1 h; norepinephrine did not change significantly

8. Conclusion

The mammalian non-visual photo-receptive system is engaged by blue LED light in a different manner than red; blue appears to be the stronger among the two types of light to provide suppression of nocturnal melatonin production and circadian phase shifting, while red is relatively protective in terms of melatonin production and circadian stability. In humans and rodents, poultry and cattle, the slight imaging differences are followed by measurable differences in immune function, other physiological parameters and the stress hormones, cortisol and norepinephrine, albeit with differing patterns and levels depending on the species, dose or timing of exposure and outcome measured.

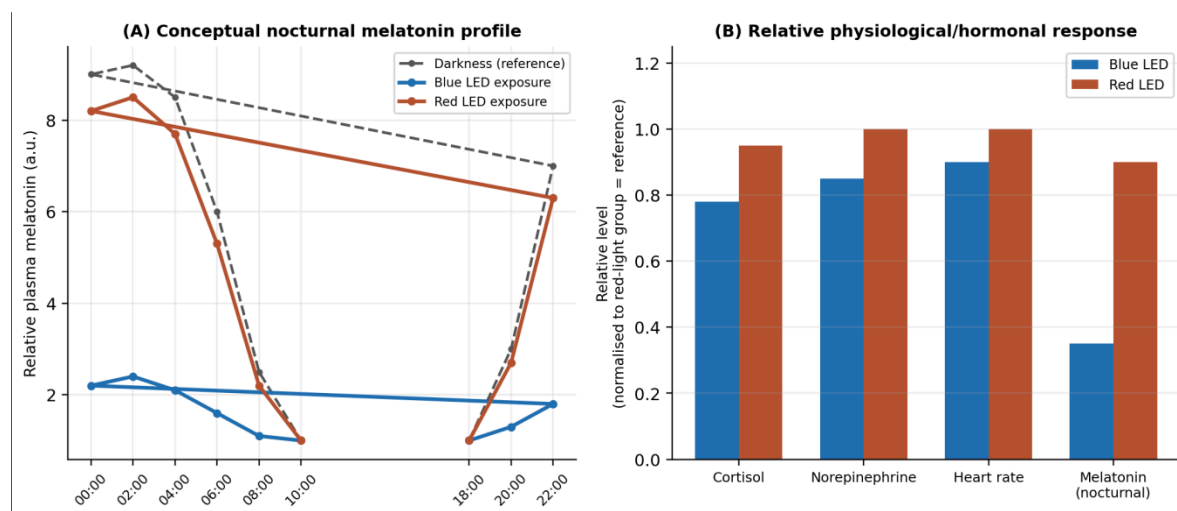


Figure 2. Conceptual comparison of blue versus red LED lighting effects. (A) Idealised nocturnal melatonin profile under darkness, blue LED exposure, and red LED exposure, illustrating the stronger suppressive effect of blue light. (B) Relative physiological and hormonal responses under blue versus red LED exposure

Research gaps and future directions

This review draws attention to a considerable disconnect between the number of human and rodent and poultry studies comparing light effects with blue lights and red lights, versus the comparatively few sheep direct light effect studies for both blue and red lights. The following are a few research priorities that were suggested by this gap.

- **Wavelength matched trials:** Broad band blue and red LED light compared to darkness or broad-spectrum light should be used to directly quantify the sheep's responses of melatonin, cortisol and norepinephrine, which show variable response to specific wavelengths. There is a need for controlled, wavelength-matched trials to directly quantify melatonin, cortisol and NE responses in sheep, which have different wavelength responses.
- **Timing-dependent effects:** Given that light-colour effects seem to be a strong function of whether exposure takes place in the photophase or the scotophase, future ovine studies should systematically manipulate the timing as opposed to a single exposure window.
- **Immune outcome measures:** Direct assessment of lymphocytes response to proliferation under blue and red lighting regimens, antibody titre after vaccination and oxidative stress in-mêmes, would help to determine if the immune improvements seen with melatonin receptor activation in poultry are equivalent in sheep.
- **Light colour effects vary between a life-stage for neonatal lambs, growing lambs and adult breeding ewes and rams, and between housed, pastured, and transported animals;** research needs to be conducted separately for each subpopulation instead of farm-averaged effects.

Sheep have the basic retinal and neuroendocrine structure underlying these responses and are known to be highly responsive to manipulation of melatonin levels by photoperiod or light pulses, suggesting similar nonlinear interactions of blue and red light pulses in the species. Nevertheless, there is little direct, controlled, and ovine-specific evidence and, in studies of stress in the sheep, cortisol and norepinephrine have not been found to react in kind with each other to conditions of common stress. Time matched and wavelength matched research in lambing ewes may advance along this pathway to help close the evidence gap and have important practical applications in the design of barn lighting, transport and handling, and the promotion of sheep welfare and productivity more generally.

- **Practical fixture and flock-level studies:** Farm-scale trials of actual LED fixtures, taking into account fleece cover, housing geometry and flock behaviour should be carried out to get the mechanistic responses provided in the lab and extrapolate this to workable barn lighting specifications.
- **Combination stress-marker panels:** With the demonstrated dissociation in the response of stress hormones, particularly between cortisol and NE to common stressors one should now use multi-marker panels, such as combining catecholamines, stress hormones, HRV, and behaviour scoring, stresses to obtain a more complete picture of the animal's physiological reactions in future light-colour studies to characterize the effects of stressors.

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