Mating Behaviour and Fertility of Layer Breeders in Natural Mating Colony Cages: LED Light Environmental Effects

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Abstract

Background: Natural mating colony cages for parent-stock layer breeders, instead of conventional cages with artificial insemination, have been widely adopted by many commercial farms in China. However, the fertility rate of this system is unstable and varies greatly among different cage tiers. We speculated that the difference in fertility rate might be caused by uneven illumination because vision appears to play a central role in hens' behaviour, including mating, which is an important factor that affects the reproductive performance of hens. The objectives of this study was to investigate the effects of 4 LED light colours (white: WL, red: RL, yellow-orange: YO, blue-green: BG) with 2 light intensities (10 lux, 25 lux) in each colour on mating frequency and fertility for layer breeders in natural mating colony cages.

Results: A total of 32 identical cages were involved in the 8 treatments, with 4 replicates for each treatment and 5 males and 45 females per cage. The results showed significant effects of age, light colour, light intensity and colour-intensity interaction on the frequency of mating behaviour, integrity of matings and fertility. Overall, flocks treated with WL and RL and a higher light intensity in natural mating colony cages during the laying period showed a more frequently mating behaviour, a large proportion of relatively complete matings and a higher fertility. All components of mating behaviour and fertility declined from 30 through 40 to 50 wks of age, but no significant correlation was found between fertility and mating behaviour.

Conclusions: In summary, the results of this study illustrate that different LED light colour and light intensity influenced the mating behaviour and fertility of layer breeders. WL and RL and a higher light intensity could increase the frequency of mating behaviour, the proportion of complete matings and fertility. No significant correlation was found between fertility and mating behaviour. Such knowledge can help to understand mating behaviour in natural mating colony cages and provide a basis for the optimization of light environment regulation.

Introduction

Nowadays, conventional artificial insemination cages still account for a large proportion of layer breeders in China. However, rising concerns on poultry welfare and increasing labour costs have contributed to the requirement to employ a new alternative housing system, which was referred to as stacked natural mating colony cage system for layer breeders. Laying hens in the colony cages are caged together with roosters, and the ratio of roosters and hens is generally kept between 1:10 to 1:8 and flock size is usually maintained 40 to 100 per individual cage. Compared with conventional cages, this colony cage system has broader activity space, better satisfy the natural mating behaviour requirements of hens, and reduce the stress generated by artificial insemination and damage to the cloaca [1]. In addition, it has higher automatic extent and can save more manpower compared to conventional artificial insemination cage system. Nonetheless, this system is still in the stage of exploration and optimization, and the management measures are still relatively immature. The fertility rate is unstable and varies greatly among different cage tiers. We speculated that the difference in fertility rate might be caused by uneven illumination because vision appears to play a central role in hens' behaviour, including mating, which is an important factor that affects the reproductive performance of hens. Therefore, there is a need to identify whether this situation can be improved through light environment regulation.

Environmentally controlled layer breeder houses regulate the light environment purposefully with respect to intensity and photoperiod to guarantee eggs are produced continuously throughout the year with minimum energy costs. Unintentionally, the initial choice of luminaires also influence the spectral power distribution. Consequently, intensities may be so low as to eliminate accurate recognition of individuals or another's intent. Mating cues may then simply be lost [2]. For instance, poultry can perceive ultraviolet (UVA) radiation (320–400 nm) that are not visible to humans [3]. However, fluorescent luminaires have little output at this wavelength and incandescent luminaires have practically none. The spectral power output may affect mating behaviour if those wavelengths mediating these traits or behaviours are missing or are super abundant [4]. Knowledge about the effects of UVA light on mating behaviour of hens have been well documented. Jones et al. (2001) reported that UVA light had a significant influence on the frequency of mating behaviour and enhance the communication of sexual signal, these effects were probably mediated through reflectance from plumage and/or fleshy parts of the bird [5]. UVA light may be used to communicate differences in comb and wattle texture, which are probably more clearly seen by fowl when lit by UVA supplemented light [6]. Prescott and Wathes (1999b) also showed that the skin of hens, when photographed under UVA light only, appeared more 'textured' than when viewed in visible light, this variation may also be used as a cue in mating and more widely in social signalling [7].

Among all light sources, light emitting dioxide (LED) is a special kind of semiconductor diode, which can give monochromatic and polychromatic light. Compared to incandescent and fluorescent light, LED light has a marked longer life, specific spectrum, lower thermal output, higher energy efficiency, higher reliability and frequency, as well as lower maintenance cost [8]. Most researchers have focused on the effects of monochromatic LED light colour on the behaviour of hens. Hassan et al. (2014) mentioned that higher frequencies of ground pecking, ground scratching, and tail wagging were observed in the red light and these behaviours were less frequent in the blue light [9]. Huber-Eicher et al. (2013) indicated that hens under green light spent more time on pecking at objects and had more frequent pecking at conspecifics compared with red and white light. And hens under red lighting showed less often severe pecks or distress calls than hens under white light, with green light being intermediate [10]. Our own published research found that transforming the light colour to red or dimming the light could be regarded as an effective method to reduce the risk of feather pecking and cannibalism and alleviate the fear responses of layer breeders [1]. In addition, significant impact could be generated by monochromatic LED light colour on performance and egg quality of laying hens. For red light enhances egg production of laying hens [11], whereas hens lay larger eggs under blue or green light [12]. It is well-established that red light stimulates the development of gonads, whereas blue and green spectra have little or no effect on the activity of the reproductive organs[13, 14, 15]. However, little attention has been given to the effects of monochromatic and mixed LED light on mating behaviour and fertility of layer breeders in natural mating colony cages. Due to other environmental effects and the strain differences between hens, it is difficult to draw any firm conclusion from other experiments. The results of both wavelength and light intensity on the laying hen behaviours in other housing systems may not be applicable to this colony cage system. In addition, most layer breeder enterprises using natural mating colony cages have adopted LED lights as the light source. It is crucial to explore the effects of LED light colour and intensity on mating behaviour and fertility of layer breeders in order to provide a basis for the regulation of light environment for layer breeders in natural mating colony cages. Therefore, the objectives of this study was to investigate the effects of 4 LED light colours (white, red, yellow-orange, blue-green) with 2 light intensities in each colour on mating frequency, fertility for layer breeders in natural mating colony cages.

Methods

Animals and experimental treatments

The experiment was carried out in Huayu Poultry Breeding farm, which is located in Handan, Hebei province, China. Hy-Line Brown parent-stock pullets (n = 1440) and cockerels (n = 160) were obtained from a commercial farm and transferred into the experimental house at the age of 16 wk and randomly distributed into 32 identical natural mating colony cages (2.40 × 1.20 × 0.71 m, length × width × height) with 5 males and 45 females in each cage, which was less than the minimum guidelines for housing density provided by Europe and North America. All double-sided experimental cages were arranged in 4 rows. Each experimental cage with a floor area of approximately 576 cm^2 per bird, equipped with commercial feed (9.6 cm trough/bird) and drinking facilities (6.25 birds/nipple). However, the environment of the experimental cage was without any enrichment protocol, except for claw abrasive devices **(Figure 1)**. The feed is evenly distributed in the trough and automatically distributed 4 times a day at 07:00, 11:00, 15:00, and 19:00 to ensure birds had permanent ad libitum access to feed. All birds were provided the same standard diet, containing (g/kg; calculated) 178 CP, 4.2 Met, 8.5 Lys, 38.2 Ca, 6.5Pt and 11.4 MJ ME/kg. Eggs and manure were collected once a day through egg conveyor belts and manure belts, respectively. Average air temperature and relative humidity were maintained between 16°C and 23°C, 50% and 80%, respectively, during the experiment. All birds were subjected to the same standard management regime throughout the experiment.

Eight treatments were compared in a factorial arrangement, including 4 LED light colours and each at two light intensities, and giving 4 replicate cages for each light treatment. As shown in **Figure 2**, the 4 LED light colours were i) red LED light (**RL**), at a peak wavelength (λp) of 660 nm and a dominant wavelength (λd) of 641 nm, half band width (△λd) of 20 nm; ii) yellow-orange LED light (**YO**), λp = 616 nm, λd = 600 nm, △λd = 38 nm; iii) blue-green LED light (**BG**), λp = 445 nm, λd = 479 nm, △λd = 21 nm; iv) white LED light (**WL**), λp = 449 nm, λd = 491 nm, △λd = 23 nm. All LED light lamps (Huazhaohong Optoelectronic Technology Co. Ltd., Wuxi, China) were attached to the two sides of the cage celling. Randomized block design was adopted for this experiment. These 4 rows were designated as 4 blocks. For each block, the distribution of 8 treatments were arranged according to the random number generator, as shown in **Figure 3**. Voltage for red, yellow-orange, blue-green, and white LED was adjusted based on the relative spectral sensitivity curve indicated by Prescott and Wathes [6], to ensure that the 4 lightings appeared iso-illuminant to hens. Light intensity was measured at the level of birds' heads using a precision luminometer (SRI-PL-6000, Shang Ze Photoelectric Co. Ltd., Taiwan, China) with a resolution of 0.01 lux according to human spectral sensitivity. The two light intensity were 25 lux (**HLI**) and 10 lux (**LLI**) respectively. Adjacent experimental cages were separated by shading curtains to avoid light pollution form different light colours. During the experiment period, the lighting rhythm was adjusted based on the different age phase, with a starter 8-h light at the age of 16 and 17 wk, 10-h light at the age of 18 wk, and then increased stepwisely each week to reach 16-h light at the age of 30 wk.

Behavioural observation

Direct observations were conducted on 4 consecutive days during the age of 30, 40, and 50 wks, respectively, by 2 trained people from an elevated seat in the corridor that allowed a panoramic view of the experimental cages. Each observer observed the 2 adjacent cages simultaneously in the morning and another two in the afternoon. Observation principles were brought into correspondence with each other before observation and the inter-observer agreement was frequently assessed by Kappa value during date collection to ensure the reliability of the results of behavioural observation. Treatments, replicates and time of day (morning and afternoon) of the observation were randomized as a Latin square design over the 4 days in each week. All observers observed an equal number of cages from each treatment (**Table 1**). Data of mating behaviour were collected between 08:00 and 12:00, 14:00 and 20:00 on the measurement days. The observers sat in position and allowed 10 min for the birds to habituate to the observer's presence and settle down. Descriptions of behavioural elements recorded were shown in **Table 2**. Frequencies of all components of mating behaviour for all male-female in each experimental cage were recorded on check sheets over 10 h.

Given that 5 males were confined with females in a very limited space, aggressions might be frequently observed between birds. Therefore, we also recorded feather pecking behaviour, aggressive behaviour, plumage condition, and mortality from cannibalism in another published research using the same animals as the present experiment [1].

Integrity of mating behaviours

Evaluation for integrity was conducted after behavioural observation of mating behaviour at the age of 30, 40, and 50 wks. According to the field observation and literature research, we divided the mating behaviour into 5 components: courtship display, mounting, treading, cloacal contact, and ejaculation. Courtship display involved any one of the 3 display behaviours. Although it is not always possible to see the actual cloacal contact, there are some good indicators that this has taken place. For example, the cock always stops treading and makes a definite backwards and downwards thrust with his pelvis. Ejaculation was generally accompanied by the rooster's wing flapping and the feathers of the male were raised and the entire body shaken, and the hen nearly always gave a very characteristic high intensity feather shake immediately after the cock dismounts. The integrity of mating behaviour was evaluated according to the completion of the above 5 stages in a single mating process, a score from 0 to 4 was assigned. The details about how the scoring was done is given in **Table 3**.

Determination of fertility

On weeks when mating behaviour was determined, all eggs weighted between 45 g and 78 g, excluded uncracked and deformed eggs from each experimental cage are suitable for setting were incubated. The incubator temperature was set at 38.3°C and the relatively humidity was maintained at 68%. They were candled at 14 d on transfer to hatcher. Eggs which were apparently infertile and eggs failing to hatch were opened to determine true fertility.

Statistical analysis

All statistical analyses were performed using linear mixed models parameterized with SPSS (IBM SPSS Statistics 22.0, Armonk, USA). Cage was used as the experimental unit and individual sample data within each of the replicate units (i.e., individual cage) were averaged before analysis, and the residuals were tested for normality and heterogeneity of variance. When data were not normally distributed and could not be solved by transformation, non-parametric statistics were used for analysis. The data were analysed with the fixed effects of light colour, light intensity, weeks of age, 2-factor and 3-factor interactions and the random effect of cage. The model equation was as follows:

$$Y_{ijkl} = \mu + LC_i + LI_j + WK_k + CA_l + LC*LI_{ij} + LC*WK_{ik} + LI*WK_{jk} + LC* LI *WK_{ijk} + \varepsilon_{ijkl},$$

where Y_{ijkl} = traits we have investigated; μ = model constant; LC_i = effect of light color (i =1 to 4); LI_j = effect of light intensity (j =1 to 2); WK_k = effect of weeks of age (k =1 to 3); CA_l = effect of cage (l =1 to 4); $LC*LI_{ij}$ = effect of interaction between light colour and light intensity; $LC*WK_{ik}$ = effect of interaction between light colour and weeks of age; $LI*WK_{jk}$ = effect of interaction between light intensity and weeks of age; $LC* LI *WK_{ijk}$ =effect of three-way interaction of light colour, light intensity, and weeks of age.

Effects in the statistical model were tested simultaneously and the effects were removed from the original model when they were not significant. When the effect was statistically different ($P < 0.05$), further analysis was performed. Frequencies of mating behaviour and scores for integrity of mating showed non-normal distributions that were not suitable for transformation, so the Mann-Whitney U test was applied for Post Hoc group comparisons. Mean comparisons were evaluated on fertility by Duncan’s Multiple Range test. Statistical significance was determined at $P < 0.05$ unless otherwise stated. In addition, a Spearman rank-order correlation was performed to determine correlations between mating behaviour and fertility.

Results

Behavioural traits

The statistic for inter-observer agreement, Kappa value was 0.92, 0.91 and 0.94 for wks 30, 40, and 50, respectively. These high values show that inter-observer reliability was excellent.

The influence of LED light colour, light intensity, and their interaction on the frequencies of mating behaviour for all combined wks are shown in **Table 4**. In comparison with BG group, males in the WL and RL groups had higher frequency of wing flapping and copulation ($P < 0.05$), and no significant difference was found between YO group and BG group for the frequency of wing flapping and copulation. Compared with the males in YO and BG groups, the WL and RL significantly increased the frequency of tidbitting and treading ($P < 0.05$). Males caged in WL and RL mounted more frequently than YO and BG groups ($P < 0.05$). The frequency of forced copulation in BG group was highest ($P < 0.05$), and no significant difference was found between other groups. The occurrence of waltzing was expressed more frequently in WL and YO groups than RL and BG groups ($P < 0.05$). A significant effect of the light intensity on wing flapping ($P = 0.029$), waltzing ($P = 0.013$), and tidbitting ($P = 0.013$) was found. Compared with LLI, HLI significantly increased the frequency of courtship display ($P < 0.05$). In addition, a significant light colour × intensity was noted for all mating behaviour ($P \leq 0.036$). It appeared to the males in each two combination groups of WL and RL showed more frequency of courtship display and mating.

Figure 4 shows the frequencies of courtship displays (wing flapping, waltzing, tidbitting) and mating behaviours (mounting, treading, full copulation) at the 3 ages. In general, the effects of age were highly significant, with the frequency of displays and matings showing an evident decline with age ($P \leq 0.01$). We noted that the combinations of WL and RL groups had a larger decline in the frequencies of displays and matings than other light treatments. Furthermore, the decline of occurrence of all mating behaviour from 30 to 40 wks was noticed larger than the decline from 40 to 50 weeks.

Evaluation for integrity of mating behaviours

Table 5 presents the influence of LED light colour, light intensity, and their interaction on the proportion of scores for integrity of mating behaviour for all combined wks. There was a significant effect of light colour on the proportion of scores except score 4 ($P \leq 0.036$). In comparison with BG and YO groups, RL had significantly reduced the proportion of score 0 ($P < 0.005$), while no significant difference was found between WL, YO and BG groups, neither no significant difference was found between WL and RL group for the proportion of score 0. The proportion of score 1 for RL group was lower than other groups ($P < 0.001$), and BG group likewise showed a lower proportion of score 1 than YO group ($P < 0.05$). For the proportion of score 2, YO and BG groups were higher than that in WL and RL groups ($P < 0.05$). Moreover, RL group had the highest proportion of score 3, whereas YO and BG groups had the lowest proportion of score 3, and intermediate for WL group ($P < 0.05$). In addition, only significant effect of light intensity was found on proportion of score 3 ($P < 0.005$), compared with LLI, HLI significantly improved the proportion of score 3 ($P < 0.005$). Significant colour × intensity interactions were noted for the proportion of scores except score 4 ($P \leq 0.018$). Overall, for the proportions of score 0, score 1 and score 2, treatments of RL was the lowest, and followed by the WL treatments. However, RL had the highest proportions of score 3 and score 4, and was followed by WL treatments as well.

The proportion of scores for integrity of mating behaviour varies with the ages was presented in **Figure 5**. The effects of age on the integrity of mating behaviour was highly significant ($P < 0.01$). Overall, the proportions of score 0 (augment: 4.1%, $P < 0.005$) and score 3 (augment: 5.2%, $P < 0.001$) increased significantly, and the proportion of score 4 slightly increased but not statistical, from the age of 30 wk to 50 wk. We noted that no significant fluctuation in the proportion of score 1, while the proportion of score 2 (decline: 7.1%, $P < 0.001$) had a significant downward trend with the ages. It was noticed that the integrity of mating behaviour was dominated by proportion scored 3 at different ages (> 66.2%).

Fertility and correlations with behavioural traits

As shown in Table 5, the fertility was significantly affected by light colour ($P = 0.037$), light intensity ($P = 0.044$), and their interaction ($P < 0.005$). Compared with YO and BG groups, WL and RL had a higher fertility ($P < 0.05$). In addition, in comparison with LLI group, HLI significantly improved the fertility ($P < 0.05$). During all light treatments, RL × HLI and WL× HLI had the highest fertility, whereas YO × LLI and BG × LLI were the lowest, and intermediate for RL × HLI, WL× HLI, YO × HLI, BG × HLI groups ($P < 0.05$). Estimates of fertility for different light treatments at three ages are presented in **Figure 6**. A significant effect of age was noted on fertility of all light treatments with the exception of BG × LLI group ($P < 0.05$), which constantly maintained at a relatively low level. In general, the fertility increased from 30 wk to 40 wk of age, and then declined from 40 wk to 50 wk of age, and the fertility at the age of 50 wk was lower than that at the age of 30 wk.

Spearman rank-order correlation coefficients among mating behaviour and fertility at the three different ages for all combined light treatments are given in **Table 6**. No correlations are shown between wing flapping, waltzing, tidbitting, and mounting behaviour at the 3 ages. Treading behaviour showed a significant positive correlation with mounting behaviour at the 3 ages ($P < 0.05$). A significant positive correlation was found between copulation and wing flapping, tidbitting, mounting and treading behaviour at the age of 30 wk ($P < 0.05$), while at the age of 40 and 50 wk, copulation only correlated positively with mounting and treading behaviour ($P < 0.05$). Forced copulation positively correlated with mounting and treading, and negatively correlated with copulation at the age of 30 and 40 wk ($P < 0.05$). However, only a significant negative correlation was noted between forced copulation and mounting behaviour at the age of 50 wk ($P < 0.01$). Out of our expectation, only at the age of 30 wk, a significant positive correlation was found between fertility and copulation ($P < 0.05$). However, fertility showed no clear correlation with all mating behaviour at the age of 40 and 50 wk.

Discussion

Studies have indicated that visual aspects play a vital role in the transmission and communication of sexual signals, therefore, the colour balance or spectral power distribution is an important factor affecting mating [5]. Courtship display reflect the willingness of male-female mating and generate an influence on mating frequency, is an important signal-response sequence of mating behaviour [16]. Courtship display frequency of male as the basis for mate choice in females typically performed at higher rates by sexually inactive males than do sexually inactive males [17]. The results of the present experiment showed that courtship displays were performed significantly more frequently by males confined in WL and RL, with the exception of waltzing display. Thus, males caged in YO and BG light were motivated to copulate, but were not communicating effectively with females. High frequency of forced copulation and unsuccessful mating could be attributed to deficiencies in courtship displays, especially in wing flapping and tidbitting, which attracts females to the male [18]. Millman et al. (1996) also suggested that deficiencies in cockerel courtship behaviour may contribute to the hens' lack of arousal and hence a decline in crouching [19]. Cockerels would then adopt forced copulations as a mating strategy. Alternatively, the cockerels may have learnt that they could then use their superior strength to force a hen to submit or intensive. These results were in agreement with the present experiment, which suggested that males exhibited forced copulation more frequently with the shortages of courtship display lit by YO and BG light. In addition, Craig et al. (1977) found evidence that females with early sexual maturity later became more submissive to males during mating [20]. Our unpublished studies have shown that females became sexually mature much earlier when illuminated by WL (121.75 d) and RL (119.75 d) compared with YO (126 d) and BG light (127.25 d). This could also account for why WL and RL reduced the frequency of forced copulation. However, waltzing presented different trend from other courtship behaviours, males performed less frequency of waltzing when exposed to RL and BG. This result was in consistent with Cheng et al. (1985) who indicated that waltzing in males is both aggressive and sexual, males waltzed significantly more frequently in the morning when females were least willing to crouch, suggesting that these waltzes may have large agnostic components [21]. Millman et al (2000) also concluded that waltzing in broiler breeder reflected a stronger aggressive motivation than sexual motivation [22]. Relationship of androgen metabolism and hypothalamic region of the brain indicate that links between copulatory and aggressive motivation are likely [23]. This assumption is confirmed by the greater aggressive behaviour displayed by males when lit by WL and YO light in our previous study which aimed to investigate the effect of LED light colour and light intensity on agnostic behaviour [1]. Frequently courtship displays and matings expressed by males lit by WL and RL might arise from the following causes. The WL and RL environment perhaps made the hens more attractive. For instance, a hen exposed to RL or red enriched light may have showed an image to the males which was more akin to that which it progenitor species would have evolved to recognize. WL and RL possibly increased the reflectance from plumage or fleshy parts of the bird, made the plumage, comb and wattle texture might be more distinctly seen by the birds, and enhanced the communication of sexual signal. Intuitively, if very little or no red was present in the light spectral distribution of the LED used, naturally flesh parts of the body such as combs cannot reflect the wavelength corresponding to red. Thus, combs would appear to the hens to have a totally different color. Alternatively, male libido is one important factor influencing mating frequency. Males with high libido mated more frequently than males with low libido [24]. The WL and RL environment may have had a physiological effect increasing the libido of the cockerels or hens. However, it is still unclear that the larger decline in the frequencies of displays and matings lit by WL and RL than other light treatments and we had no accurate knowledge of the relationship between light colour and sexual communication. Further studies are needed, a closer and more elaborate investigation into transmission of sexual signalling between cockerels and hens is required to understand fully the complexities of visual aspects of mating behaviour.

In the present experiment, the integrity of mating behaviour for all light treatments was dominated by proportion scored 3 at different ages (> 66.2%). This suggested that during the majority of the male-female mating behaviour, the males only completed the components of mounting, treading, cloacal contact and ejaculation, but did not commence attracting the hens to mate voluntarily by the behaviour of waltzing, wing flapping and tidbitting. In the present study, it was noticed that the proportion of complete matings was the lowest at the age of 30 wk, whereas the mating frequency was the highest. This indirectly suggested that the libido of males to copulate with females was higher during the egg-laying peak period. However, the possible large interference existed during the mating process caused low integrity of mating. On the one hand, the high stocking density in intensive farming was considered as an important contributor to the integrity of displays and matings [25]. On the other hand, during domestication and breeding, the birds was produced mainly by artificial insemination, more emphasis is placed on their production performance than on natural behaviour requirements, this may also result in their offspring having incomplete mating behaviour than the jungle fowl [26]. In addition, the early rearing history of males and females has important influence on the expression of later mating behaviour as well. Studies have shown that birds must learn the characteristics of appropriate mates for normal sexual activity, this learning, termed as sexual imprinting, is most likely to form during a sensitive period prior to sexual maturity, which is about 10–12 wks of age [27]. Rearing males and females separately or males kept in captivity in all-male groups can cause problems with breeding, will generate strong homosexual pair bonds and decreased or less complete mating behaviour when later mixed with females [28, 29]. The parent-stock of the layer breeder used in the present experiment was produced by artificial insemination, meanwhile, the cockerels and pullets used were reared separately for independent control of body weight, this could result in incomplete mating and reduced fertility. Furthermore, high-ranking males yielded most of the offspring [30], females sustain closer proximity to high-ranking males, and preferentially solicit copulations from them [31]. Males usually interfere with the mating of males subordinate to themselves. Mating by low-ranking males can become almost completely suppressed [32]. Therefore, high stocking density in a natural mating colony cage makes the mating behaviour of the low-ranking males more urgent. In addition, the high stocking density and limited space would also cause other subordinate males to frequently appear in the territory of the high-ranking males, which made their mating behaviour more competitive. These could be responsible for the high proportion of incomplete mating behaviour in the natural mating colony cages.

Beyond expectation, a surprising finding of the present experiment was that the absence of a directly correlation between fertility and mating behaviour at each age. The large decline in all elements of mating behaviour from 30 through 40 to 50 weeks of age is strongly suggestive of a decrease in libido. Nevertheless, this did not contribute to a general decrease in fertility. Perhaps it is because the males were inseminating the females more frequently than necessary to sustain a high level of fertility. This indicates that a portion of the mating behaviour that was recorded early in this experiment was either superfluous to demands or was not as integrated as had been thought. The consequences of the present study validated previous researches that mating behaviour would not be closely related to fertilizing capacity in poultry and the reduced fertility was not caused by differences in the fertilizing capacity of their semen, but due to their failure to copulate with as many hens [33]. This suggests that the decline in fertility that is, however, associated with other age-related changes in mating behaviour [18]. Although there was no direct relationship of fertility and mating behaviour, the results from the present experiment indicated that the patterns of mating behaviour and fertility for different light treatments were similar. These results were confirmed by Soley and Groenwald (1999) who indicated that abnormalities in mating behaviour should be responsible for poor fertility [34]. Kirk et al. (1980) also pointed out that in breeding poultry, fertility is often higher early in the laying period, but declines later, especially after 50 wks of age [35]. This decline has been attributed to males rather than females, because fertility can be maintained by artificial insemination [36]. However, this perspective was not entirely accepted by us. It has been hypothesized that the large decline in all elements of mating behaviour in the present study was arose from the decline in libido in the males, nonetheless, the probability occurs that female receptivity might have been involved in the males' reaction. Although research of female mating behaviour in hens have not been widespread, the problem of low fertility was mainly because of a low mating frequency in the female as well as in the males. In this particular case, male and female behaviour seem to contribute equally to fertility. For instance, Moyle et al. (2010) proposed that feather loss on the females’ back could generate an effect on the willingness of males to copulate, and females with more feather loss, especially as they are ageing, will have lower fertility than females with more feather covering on their backs [37]. Therefore, except for the declined libido of males, perhaps the deteriorated back feather covering of females could also account for the reduced frequency of mating behaviour and fertility with age. Furthermore, in layer breeder flocks for natural mating colony cages, males or females may have to be added to the cages if many of them die or have to be culled, so maintaining the sex ratio. There is also a practice, in which males or female will be exchanged among different cages about mid-way through the laying period in an attempt to reduce the decline of fertility. It is believed that this practice elevate the freshness between males and females, and increase the frequency of mating behaviour both directly, by themselves mating, and also indirectly, by stimulating the activity of the resident. Although no publications has demonstrated on the availability of this management practice, based on informal observations by employees, the new males or females implemented a higher proportion of matings than would have been expected from their numbers. In this case, we suspected that the decrease in motivation to copulate or fertility might be due to the increasing of aesthetic fatigue or habituation to stimulus female with age. The claw removal of toes from males in this natural mating colony cage to prevent scratching of the females can also contribute to decreased mating efficiency and fertility in these males as they are ageing.

In general, a higher fertility was found for WL and RL when lit by a brighter illumination in the present study. It has been assumed that the libido of males to copulate, the mating frequency and other behaviour related management were the main factors affecting fertility. Therefore, the higher frequency of mating behaviour, large proportion of complete and effective copulation in WL and RL might contribute to the higher fertility. Campo and Davila (2002) investigated the influence of mating ratio and group size on indicators of fearfulness and stress of hens and cocks and indicated that the high sex ratio of males and females could increase psychological and physical stress in both males and females, and the main cause of stress is an increase in the number of males [38]. Marin and Satterlee (2003) studied the effects of adrenocortical responsiveness on males' mating behaviour in Japanese quail, suggested that males were more likely to mate females that are less stressed [39]. Ottinger and Mench (1989) also indicated that crowding and large group sizes should be avoided, partly because stress can reduce mating frequency and depress fertility ability [40]. Our previous study have indicated that the overall frequency of feather pecking and stress level was lower in WL and RL than when lit by YO and BG [1]. Therefore, the dominant ranking of males is more obvious, and the competition and interference during mating is reduced. Meanwhile, the stress level of hens is lower, so the frequency of mating behaviour, complete mating and fertility were elevated. An interesting question is raised by the finding of such a high level of mating in wk 30 compared with the much lower level in wk 40, which was sufficient to maintain the highest level of fertility. This was mainly because the effective and maximum duration of fertility were at 36 wks of age, and the ability of hens to store viable sperm could maintain the maximum fertility last for 2 wks [41]. The measured time of wk 40 in the present experiment was within the above range. The results of the initially low and general decline from 40 to 50 wks of age in fertility also suggested that the existence of superfluous copulatory behaviour early in the present study, and the possibility that some of these copulations may have been not effective, also suggested that this behaviour may have some function apart from fertilization, such as improving the cohesiveness of the social group.

Conclusions

In summary, the results of this study illustrate that different LED light colour and light intensity influenced the mating behaviour and fertility of layer breeders. Birds treated with WL and RL and a higher light intensity in natural mating colony cages during the laying period showed a more frequently mating behaviour, a larger proportion of relatively complete matings and a higher fertility. All components of mating behaviour and fertility declined from 30 through 40 to 50 wks of age, but no significant correlation was found between fertility and mating behaviour. Such knowledge can help to understand mating behaviour in natural mating colony cages and provide a basis for the optimization of light environment regulation. In addition, we established the scoring system to evaluate the integrity of mating behaviour and this was feasible but still need to be improved. However, the other related behavioural and management factors affecting mating behaviour and fertility, for instance, male-male and male-female aggression, feather condition, stress level, body weight, more light colour, a broader range of light intensity and so on should be involved in further study. Moreover, we still had no accurate knowledge of the relationship between light colour and sexual communication. Further studies are needed, a closer and more elaborate investigation into transmission of sexual signalling between cockerels and hens is required to understand fully the complexities of visual aspects of mating behaviour.

Abbreviations

LED: Light emitting diode; WL: White light; RL: Red light; YO: Yellow-orange light; BG: Blue-green light; HLI: High light intensity; LLI: Low light intensity; UVA: Ultraviolet A; WF: Wing flapping; FC: forced copulation; Wks: Weeks of age.

Declarations

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Authors' contributions

WZ and BL conceived and designed this study. HS performed experiments, analyzed data and wrote the manuscript. QT assisted with data analysis and paper writing. DZ provided the experimental house and hens. WZ and BL contributed to the revisions. All authors reviewed and approved the final manuscript.

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Availability of data and materials

All data generated or analyzed in this study is available from the corresponding authors upon reasonable request.

Ethics approval

All birds were managed by trained staff and the procedures relating to the use of live birds in this experiment were approved by The Laboratory Animal Ethical Committee of China Agricultural University.

Consent for publication

All of the authors have approved the final version of the manuscript, agree with this submission to Journal of Animal Science and Biotechnology

Competing interests

The authors declare that they have no competing interests.

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Tables

Table 1

The order of behavioral observation was randomized as a Latin square over each wk

Day	AM 8:00-12:00				PM14:00-20:00			
Day 1	Observer A	RL1	Observer B	YO1	Observer A	BG1	Observer B	WL1
		RL2		YO2		BG2		WL2
Day 2	Observer B	BG1	Observer A	WL1	Observer A	RL1	Observer B	YO1
		BG2		WL2		RL2		YO2
Day 3	Observer B	WL1	Observer A	RL1	Observer A	YO1	Observer B	BG1
		WL2		RL2		YO2		BG2
Day 4	Observer A	YO1	Observer B	BG1	Observer B	WL1	Observer A	RL1
		YO2		BG2		WL2		RL2
Note: WL = white, RL= red, YO = yellow-orange, BG = blue-green; 1 = 25 lux, 2 = 10 lux.								

Table 2

Definition of recorded mating behaviours

Behavioural category	Behaviour	Description
Courtship display	Wing flapping (**WF**)	Both wings of the male are raised above the level of the back and flapped
	Waltzing	The male moves round the hen with short shuffling steps and drops the wing farthest from the hen
	Tidbitting	The male pecks and scratches at the ground and gives food calls
Mating	Mounting	The male approached a female and placed one or more feet on her back. The female avoided the male, and no further elements of the copulatory sequence were observed.
	Treading	The male makes small treading movements with his feet and depresses and spreads his tail to one side of the hen's tail, and no further elements of the copulatory sequence were observed.
	Copulation	The male mounted, gripped, and trod a female and appeared to achieve cloacal contact and ejaculation. The female ruffled her feathers following the male's dismount.
	Forced copulation (**FC**)	The male mounted a female and appeared to achieve cloacal contact following a struggle, during which the female attempted to avoid the male. The female often without crouching and squawked during the struggle.

Table 3

Description of scoring method for integrity of mating behaviours of males

Score	Description
0	The male does not display courtship[1], cloacal contact[2], ejaculation[3] and treading, but mounting are showed
1	The male does not display courtship, cloacal contact[2] and ejaculation[3], but mounting and treading are showed
2	The male does not display courtship and ejaculation, but makes mounting, treading and cloacal contact
3	The male does not display courtship, but makes mounting, treading, cloacal contact and ejaculation
4	All components are fully expressed

Note: [1]Courtship displays: courtship involved any one of the three display components (wing flapping, waltzing, tidbitting); [2]Cloacal contact: the cock always stops treading and makes a definite backwards and downwards thrust with his pelvis; [3]Ejaculation: ejaculation was generally accompanied by the rooster's wing flapping and the feathers of the male were raised and the entire body shaken, and the hen nearly always gave a very characteristic high intensity feather shake immediately after the cock dismounts

Table 4

Influence of LED light colour, light intensity, and their interaction on the frequencies[1] of mating behaviour during 10-h observation periods for all wks combined*.

Treatment	Courtship display			Mating			
	WF[4]	Waltzing	Tidbitting	Mounting	Treading	Copulation	FC[5]
Light colour[2]							
WL	2.01^{ab}	1.11^{a}	0.68^{a}	1.91^{a}	1.64^{a}	1.36^{ab}	0.37^{b}
RL	2.15^{a}	0.74^{b}	0.61^{a}	1.87^{a}	1.66^{a}	1.51^{a}	0.32^{b}
YO	1.78^{bc}	1.06^{a}	0.44^{b}	1.71^{b}	1.42^{b}	1.23^{bc}	0.41^{b}
BG	1.59^{c}	0.82^{b}	0.36^{b}	1.56^{c}	1.36^{b}	1.05^{c}	0.56^{a}
Pooled SEM	0.14	0.12	0.06	0.14	0.12	0.15	0.03
Light intensity[3]							
LLI	1.69^{b}	0.81^{b}	0.49^{b}	1.69	1.48	1.21	0.42
HLI	2.03^{a}	1.02^{a}	0.60^{a}	1.77	1.54	1.38	0.40
Pooled SEM	0.06	0.09	0.05	0.12	0.10	0.13	0.02
Colour-Intensity							
WL-HLI	2.02^{ab}	1.21^{a}	0.73^{a}	1.86^{ab}	1.64^{a}	1.42^{ab}	0.32^{b}
WL-LLI	1.92^{bc}	1.03^{ab}	0.64^{ab}	1.74^{bcd}	1.61^{a}	1.26^{bc}	0.39^{b}
RL-HLI	2.24^{a}	0.87^{bcd}	0.71^{a}	1.98^{a}	1.72^{a}	1.57^{a}	0.34^{b}
RL-LLI	2.12^{ab}	0.64^{d}	0.66^{ab}	1.72^{bcd}	1.58^{ab}	1.48^{a}	0.29^{b}
YO-HLI	1.99^{bc}	1.16^{a}	0.51^{bc}	1.76^{bc}	1.43^{bc}	1.39^{ab}	0.41^{b}
YO-LLI	1.65^{d}	0.94^{bc}	0.37^{cd}	1.67^{cd}	1.42^{c}	1.13^{cd}	0.39^{b}
BG-HLI	1.82^{cd}	0.82^{cd}	0.44^{c}	1.54^{d}	1.36^{c}	1.14^{cd}	0.59^{a}
BG-LLI	1.58^{d}	0.74^{d}	0.28^{d}	1.61^{d}	1.31^{c}	0.95^{d}	0.58^{a}
Pooled SEM	0.14	0.13	0.08	0.16	0.14	0.21	0.07
F statistics							
Light colour	2.23	3.26	2.25	3.39	2.75	3.81	2.54
Light intensity	2.41	2.58	2.41	0.92	1.73	1.82	0.67
Colour × Intensity	3.68	3.32	2.97	3.21	2.34	5.53	2.41
Source of variation							

Light colour	0.034	0.006	0.042	0.005	0.012	0.004	0.018
Light intensity	0.029	0.013	0.036	0.421	0.075	0.073	0.795
Colour × Intensity	0.003	0.007	0.011	0.007	0.036	0.001	0.024

Note: *Statistical comparisons relate to the effects of light colour, light intensity and their interaction, values are means of four replicate cages with 50 birds per cage; $^{a\text{-}c}$ Means within a column and effects that lack common superscripts differ significantly ($P < 0.05$); [1]Frequency was expressed as per male per hour; [2]Light colours: WL = white, RL = red, YO = yellow-orange, BG = blue green; [3]Light intensity: HLI = 25 lux, LLI = 10 lux; [4]WF:= wing flapping; [5]FC = forced copulation.

Table 5
Influence of LED light colour, light intensity, and their interaction on the proportion of scores for integrity of mating behaviour and on fertility for all wks combined*.

Treatment	Score (%)					Fertility (%)
	0	1	2	3	4	
Light colour[1]						
WL	1.78[ab]	13.92[ab]	9.84[b]	72.69[b]	2.30	93.50[a]
RL	1.13[b]	9.37[c]	9.75[b]	76.85[a]	2.73	94.05[a]
YO	2.85[a]	14.47[a]	11.62[a]	69.40[c]	1.64	92.50[b]
BG	2.95[a]	12.96[b]	12.39[a]	70.52[c]	1.98	92.55[b]
Pooled SEM	0.21	1.44	1.32	4.12	0.22	0.42
Light intensity[2]						
LLI	2.10	12.02	10.66	72.20[b]	2.14	92.41[b]
HLI	1.66	11.30	10.03	75.34[a]	2.18	93.86[a]
Pooled SEM	0.16	1.26	1.19	3.65	0.13	0.36
Colour-Intensity						
WL-HLI	1.73[abc]	13.67[ab]	10.20[cd]	73.27[c]	2.17	94.56[a]
WL-LLI	1.82[abc]	14.17[a]	9.48[d]	72.10[c]	2.43	92.42[b]
RL-HLI	1.05[c]	8.53[d]	9.52[d]	78.20[a]	2.77	94.69[a]
RL-LLI	1.21[bc]	10.20[c]	9.97[d]	75.87[b]	2.68	93.31[b]
YO-HLI	3.13[a]	14.53[a]	11.83[ab]	68.93[d]	1.47	93.17[b]
YO-LLI	2.57[ab]	14.40[a]	11.37[bc]	69.87[d]	1.80	91.82[c]
BG-HLI	3.11[a]	12.63[b]	12.97[a]	70.00[d]	2.30	93.00[b]
BG-LLI	2.80[a]	13.30[ab]	11.80[ab]	70.97[d]	1.67	92.10[c]
Pooled SEM	0.23	1.84	1.65	4.73	0.23	0.55
F statistics						
Light colour	2.86	2.43	1.28	1.33	1.24	2.68
Light intensity	1.25	1.01	1.12	5.23	1.16	2.54
Colour × Intensity	1.19	8.59	1.54	11.02	1.32	7.91
Source of variation						
Light colour	0.021	0.012	0.015	0.015	0.174	0.037

Light intensity	0.204	0.425	0.375	0.005	0.352	0.044
Colour × Intensity	0.018	0.001	0.013	0.001	0.084	0.005

Note: *Statistical comparisons relate to the effects of light colour, light intensity and their interaction, values are means of four replicate cages with 50 birds per cage; [a-d] Means within a column and effects that lack common superscripts differ significantly ($P \leq 0.05$); [1]Light colours: WL = white, RL = red, YO = yellow-orange, BG = blue green; [2]Light intensity: HLI = 25 lux, LLI = 10 lux.

Table 6
Spearman rank-order correlation coefficients among mating behaviour and fertility at the three different ages for all light treatments combined.

Age	Item	WF[1]	Waltzing	Tidbitting	Mounting	Treading	Copulation	FC[2]	Fertility
30	WF	1							
	Waltzing	0.221	1						
	Tidbitting	0.196	0.160	1					
	Mounting	0.185	0.117	0.145	1				
	Treading	0.153	0.102	0.081	0.454*	1			
	Copulation	0.462*	0.281	0.473*	0.712*	0.594**	1		
	FC	-0.264	-0.355	-0.240	0.544**	0.596**	-0.425*	1	
	Fertility	0.246	0.163	0.336	0.275	0.324	0.436*	0.206	1
40	WF	1							
	Waltzing	0.168	1						
	Tidbitting	0.136	0.178	1					
	Mounting	0.201	0.122	0.109	1				
	Treading	0.162	0.136	0.055	0.386*	1			
	Copulation	0.303	0.258	0.368	0.574*	0.455**	1		
	FC	-0.284	-0.263	-0.162	0.472*	0.509**	-0.545*	1	
	Fertility	0.158	0.130	0.217	0.312	0.326	0.330	0.102	1
50	WF	1							
	Waltzing	0.144	1						
	Tidbitting	0.114	0.216	1					
	Mounting	0.232	0.128	0.085	1				
	Treading	0.104	0.149	0.042	0.403*	1			
	Copulation	0.260	0.286	0.325	0.466*	0.489*	1		
	FC	-0.155	-0.172	-0.261	-0.449**	0.340	0.367	1	
	Fertility	0.240	0.182	0.191	0.275	0.160	0.204	0.185	1

Note: * $P < 0.05$, ** $P < 0.01$; [1]WF = wing flapping; [2]FC = forced copulation.

Figures

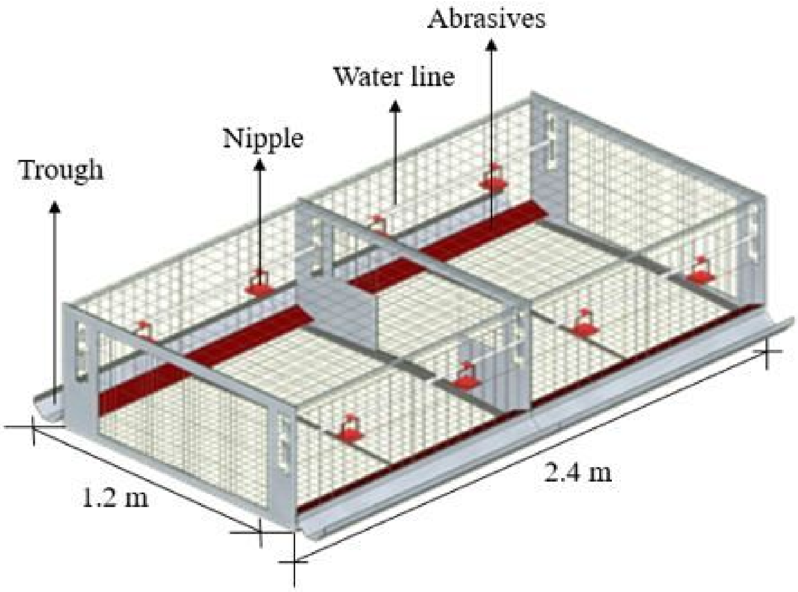

Figure 1

Schematic diagram of the natural mating colony cage.

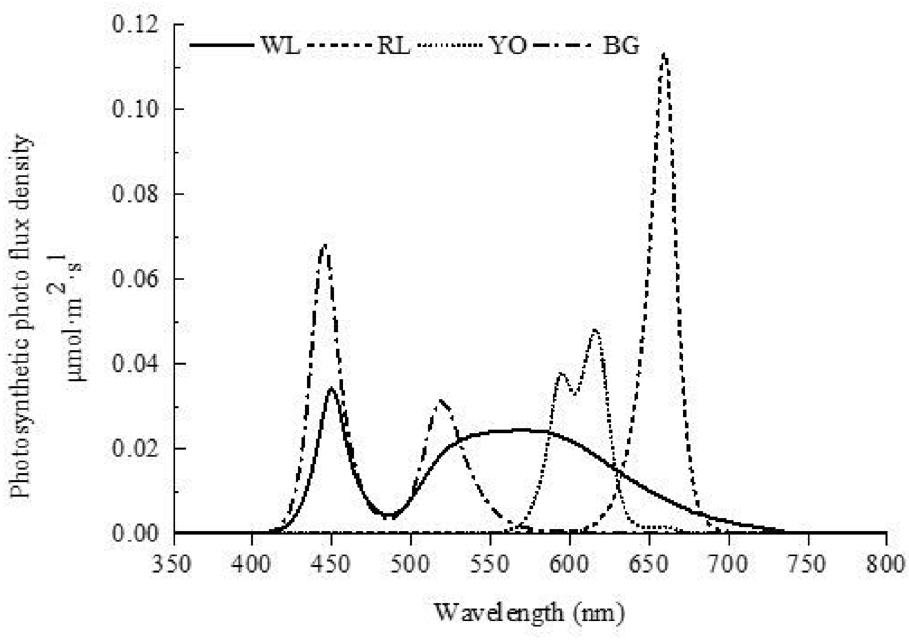

Figure 2

Light spectral distribution of four LED lights (WL: white, RL: red, YO: yellow-orange, BG: blue-green).

YO2	WL1	WL2	YO1	RL1	BG2	BG1	RL2
WL2	YO2	BG1	RL1	YO1	WL1	RL2	BG2
BG1	RL2	WL1	YO1	YO2	WL2	BG2	RL1
YO2	BG2	RL2	RL1	WL2	WL1	BG1	YO1

Figure 3

The distribution of the different LED light colour and light intensity treatments across all rows. Adjacent experimental cages were separated by shading curtains to avoid light pollution form different light colours. (WL = white, RL= red, YO = yellow-orange, BG = blue-green; 1 = 25 lux, 2 = 10 lux).

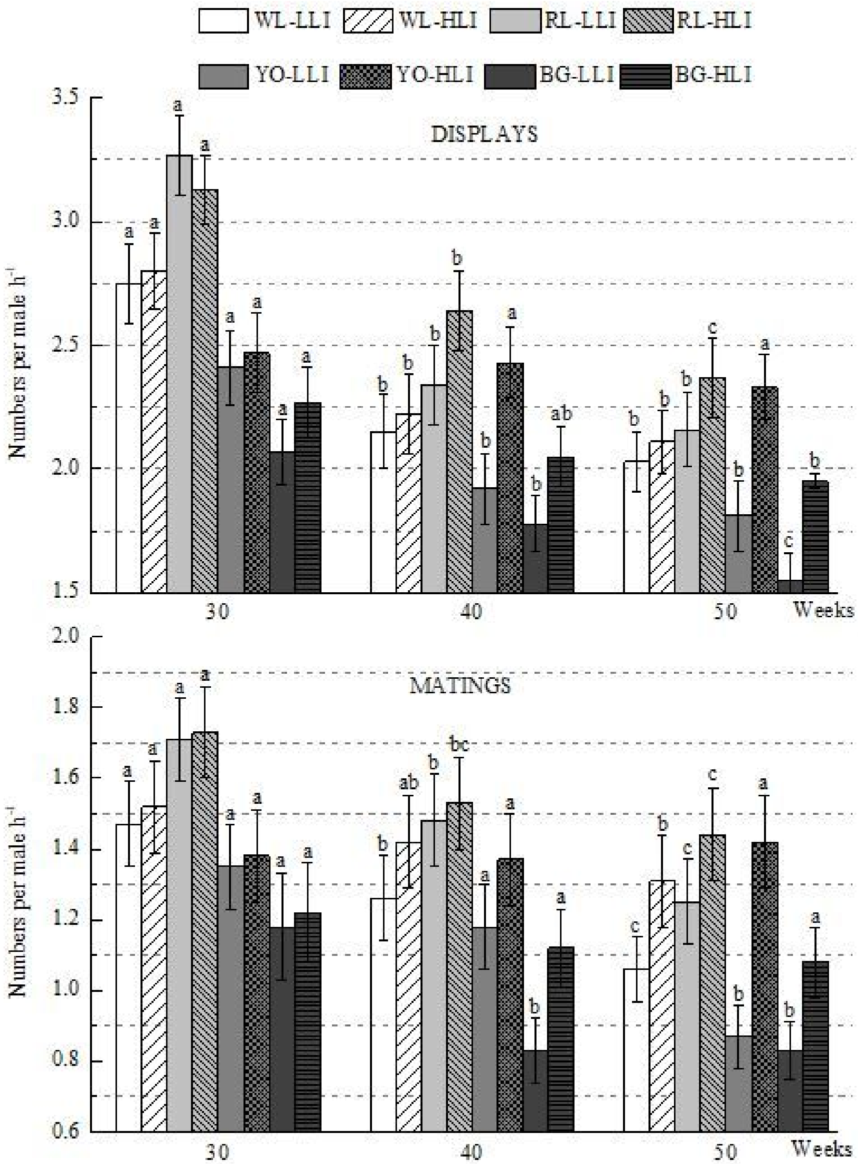

Figure 4

Frequencies of courtship displays (wing flapping, waltzing, Tidbitting) and mating behaviours (mounting, treading, full copulation) at the three ages. Statistical comparisons relate to the effects of light colour, light intensity and their interaction, the effects of light colour and light intensity were not presented. Values are means ± SE of four replicate cages with 50 birds per cage; a-c Means within a column and effects that lack common superscripts differ significantly ($P < 0.05$); Frequency was expressed as per male per hour; WL = white, RL = red, YO = yellow-orange, BG = blue green; HLI = 25 lux, LLI = 10 lux.

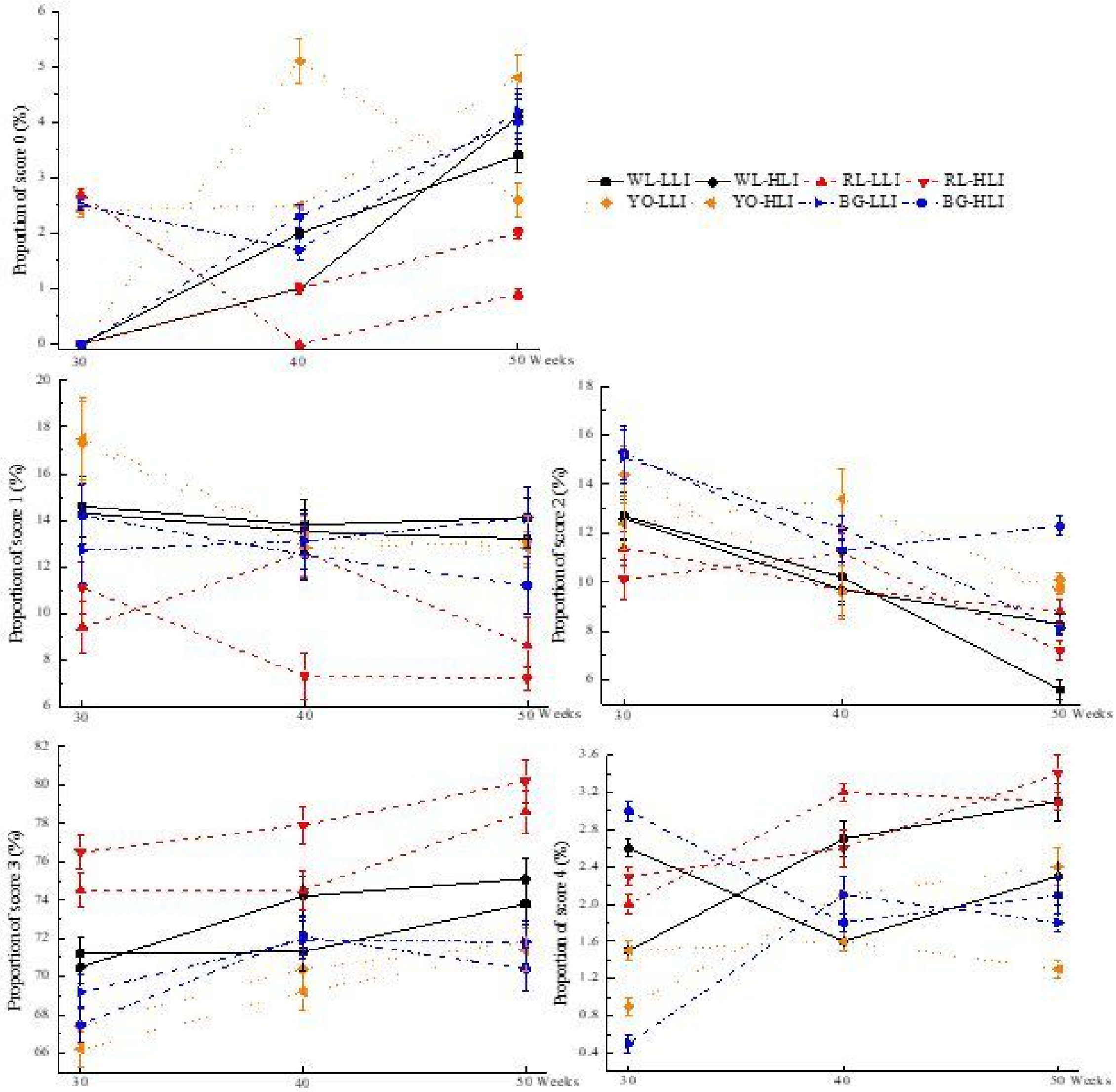

Figure 5

The proportion of scores for integrity of mating behaviour at the three ages. Statistical comparisons relate to the effects of light colour, light intensity and their interaction, the effects of light colour and light intensity were not presented. Values are means ± SE of four replicate cages with 50 birds per cage; WL = white, RL = red, YO = yellow-orange, BG = blue green; HLI = 25 lux, LLI = 10 lux.

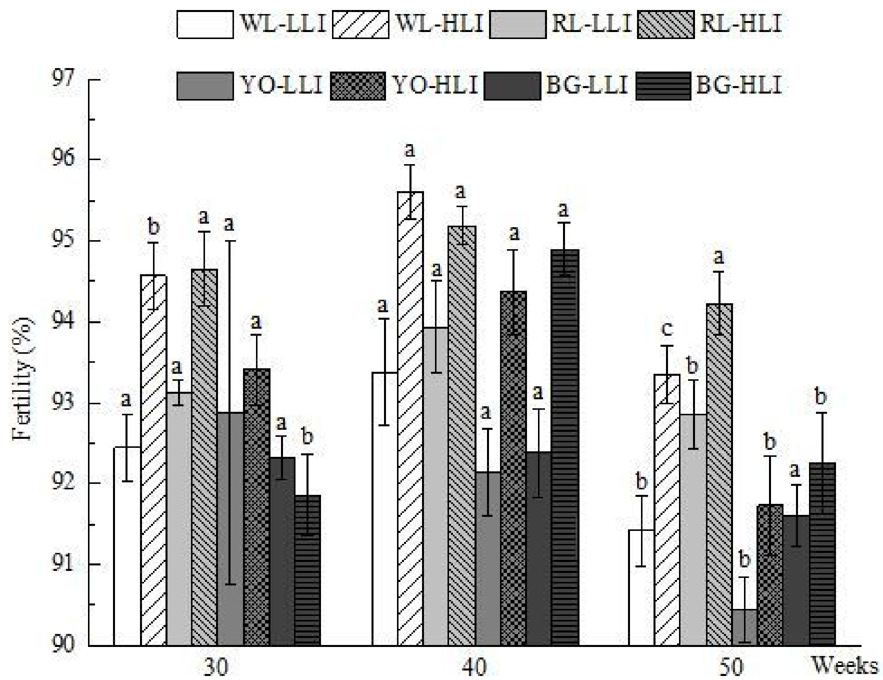

Figure 6

The fertility at the three ages for different groups. Statistical comparisons relate to the effects of light colour, light intensity and their interaction, the effects of light colour and light intensity were not presented. Values are means ± SE of four replicate cages with 50 birds per cage; a-c Means within a column and effects that lack common superscripts differ significantly ($P < 0.05$); WL = white, RL = red, YO = yellow-orange, BG = blue green; HLI = 25 lux, LLI = 10 lux