



Article

Illuminating Solutions for Reducing Mislaid Eggs of Cage-Free Layers

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Abstract: Social dynamics and lighting conditions influence floor egg-laying behavior (FELB) in hens. Hens prefer to lay eggs in darker areas, leading to mislaid eggs in cage-free systems. Consistent lighting is crucial to prevent mislaid eggs, but equipment obstructions can result in a dark floor area. These dark areas entice hens to lay their eggs outside the designated nesting area, which can lead to potential losses, damage, or contamination, creating hygiene problems and increasing the risk of bacterial growth, resulting in foodborne illnesses. Therefore, additional lighting in dark areas can be a potential solution. The objectives of this study were to evaluate the effectiveness of providing additional light in darker areas in reducing the number of mislaid eggs and FELB. Approximately 720 Hy-Line W-36 hens were housed in four cage-free experimental rooms (180 hens per room), and 6 focal hens from each room were randomly selected and provided with numbered harnesses (1–6) to identify which hens were performing FELB and identify the effect of illuminating solutions. Eggs laid on the floor and in nests were collected and recorded daily for two weeks before and after the light treatment. Statistical analysis was performed using paired t-tests for mislaid eggs and logistic regression for FELB in R Studio ($p < 0.05$). This study found that additional lighting in darker areas reduced the number of mislaid eggs by 23.8%. Similarly, the number of focal hens performing FELB decreased by 33.3%. This research also unveiled a noteworthy disparity in FELB, with approximately one-third of hens preferring designated nesting areas, while others opted for the floor, which was influenced by social dynamics. Additionally, egg-laying times varied significantly, ranging from 21.3 to 108.03 min, indicating that environmental factors and disturbances played a substantial role in this behavior. These findings suggest that introducing additional lighting in darker areas changes FELB in hens, reducing mislaid eggs and improving egg quality in cage-free systems.

Keywords: animal welfare; cage-free housing; egg production; laying hen; lighting system; mislaid eggs; mitigation strategy



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1. Introduction

Cage-free egg production has become increasingly popular as consumers demand more humane treatment of animals [1–3]. However, this new type of production system comes with new challenges. One of the significant challenges is mislaid eggs, which affect egg quality, production efficiency, and profitability [4,5]. Mislaid eggs refer to eggs laid outside the designated nesting area, leading to potential losses, damage [5,6], or contamination [7,8]. Therefore, mislaid eggs can create hygiene problems and increase the risk of bacterial growth [7], resulting in foodborne illnesses [9–11].

Previous studies have shown that mislaid eggs can account for 0.2–2% of a poultry farm's daily egg production, and, in extreme cases, this number can reach up to 10% in cage-free aviary housing [12]. However, the average weekly mislaid eggs in cage-free floor-raised housing was 31.6–59.6%, which is higher than other housing systems [13]. Mislaid eggs result from various factors. In response, numerous management strategies have been examined to prevent their occurrence. One approach involves training young

pullets to use nest boxes by providing early access to the nest boxes [14]. However, in some cases, birds may lack the necessary nesting training or have restricted access to the nest boxes, leading to floor laying. Cleaning and drying nest boxes, using soft and comfortable materials, and creating a dimly lit environment can make it more attractive for birds to lay their eggs [14,15]. Similarly, providing young pullets with a perch also helps to reduce floor eggs [16]. A delayed collection of mislaid eggs can also encourage other birds to lay on the litter floor and become habituated to this behavior [5,17]. Moreover, mislaid eggs that are not promptly collected are at an increased risk of bacterial contamination from litter or manure [7] and can be eaten or broken by other birds [5].

According to Santos et al. [18], the rapid rise in the global population and shifts in dietary preferences have brought production technology research to the forefront. Optimizing production by providing adequate environmental conditions supports producers in increasing efficiency and productive sustainability. Reducing mislaid eggs is the most challenging management aspect to ensure egg quality and safety. The Food and Drug Administration (FDA) in the United States [11] and the European Food Safety Authority (EFSA) in Europe [19] are aware of how mislaid eggs can harm consumers. That is why they mandate that food producers have strategies to prevent mislaid eggs and ensure safe products. As a result, many food producers, researchers, and other agencies have adopted approaches like regular inspections [20], employee training [20], sanitation [21], and using technology like egg detection systems to detect defective eggs [22] and make their food products safer. Recent technologies, such as automated floor monitoring systems and computer vision, have also been developed to detect mislaid eggs [17,23,24] and floor egg-laying behavior (FELB) [5]. These systems use cameras to monitor hens' FELB or detect eggs outside the designated nesting area. These technologies allow producers to identify problem areas and take corrective action, reducing the number of mislaid eggs.

The cage-free housing system, commonly used for raising egg-laying hens, has been known to cause the development of dark areas within the housing area, which might be due to various pieces of equipment such as fans, heaters, perches, drinkers, feeders, or the whole aviary structure present [25]. This equipment blocks the light and creates dark areas or spots (where the light intensity is lower than other places within the room) on the floor (Figure 1). These areas can trigger the hens to perform mislaying behavior, which refers to laying eggs in areas outside their designated nests [26]. Hens may be drawn to these areas because they perceive them as safe, secluded nest areas. However, this behavior can have negative consequences for both the hens and the farmers, resulting in decreased egg production and an increased risk of egg breakage and egg-eating behavior [5,6,27]. Therefore, farmers and researchers need to identify and address the underlying causes of mislaying behavior to ensure the health and productivity of their hens. Research on mislaid eggs in cage-free housing has been ongoing and is examining the causes, impacts, and possible mitigation solutions. One potential solution that has been explored is the provision of high-intensity lighting in dark areas of the production facility [25,26], as it encourages hens to lay their eggs in designated nesting areas. The theory was that hens lay eggs in dark areas, leading to mislaid eggs. Therefore, providing additional lighting can help to reduce the number of mislaid eggs. This research hypothesizes that providing additional lighting in darker areas decreases mislaid eggs and mislaying behaviors in hens. The objective of this study was to evaluate the effectiveness of providing additional light in darker areas in reducing (a) the number of mislaid eggs and (b) FELB.

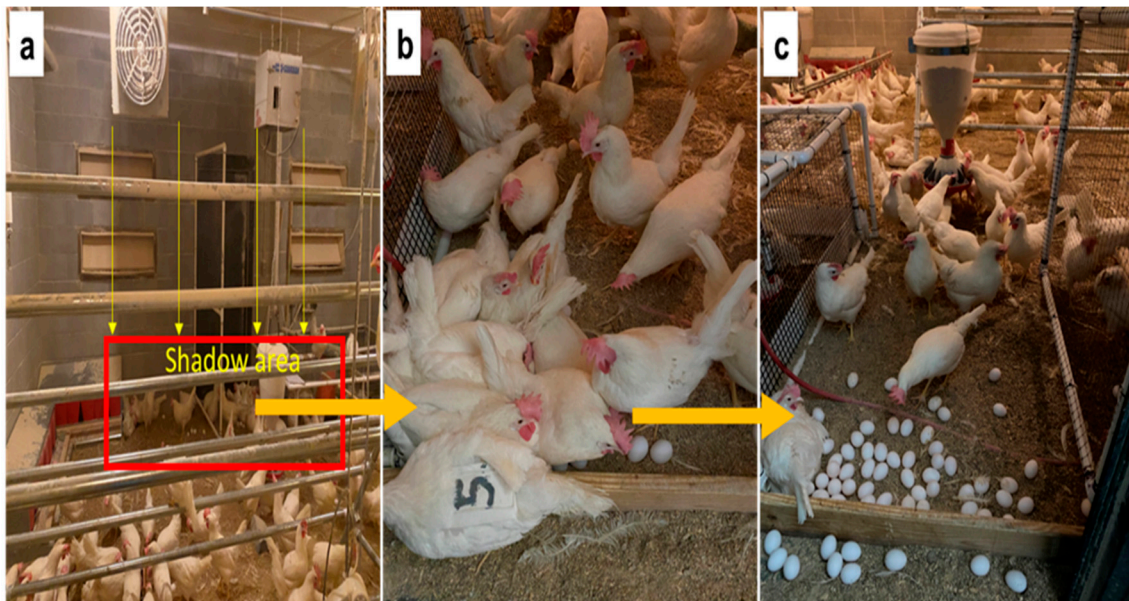


Figure 1. Experimental room showing (a) shadows from equipment that cause a dark area, (b) floor egg-laying behavior of hens, and (c) mislaid eggs on the floor litter in a darker area.

2. Materials and Methods

2.1. Ethical Approval

This study was conducted at the University of Georgia's Poultry Research Center in a facility where laying hens were raised on the litter floor. Four identical experimental rooms were used in this research, and all procedures were approved by the Institutional Animal Care and Use Committee (IACUC) prior to the start of the study (AUF#: A2020 08-014-A1, approved on 5 October 2020).

2.2. Housing and Management

Each room in the facility measured 7.3 m in length, 6.1 m in width, and 3.1 m in height (Figure 2). Each room raised 180 laying hens (Hy-line W-36) from 27 to 30 weeks of age (WOA). The litter space in each room (excluding perches and other equipment) was approximately 37.9 square meters (408 square feet), with a stocking density of 0.21 square meters of litter floor space per bird, which is higher than the recommended stocking density (minimum 1.5 square feet/hen) for commercial cage-free housing in the United States [28]. Each room consisted of pine wood shavings (with an initial depth of 2 inches) as bedding material and an A-shaped perch of 36.6 m in total length (equivalent to 0.2 m of perch space per bird). In addition, 4 nest boxes (0.71 m²; 0.016 m² per hen; 45 hens per nest box) were provided in each room at 14 WOA to make birds habituate the nesting areas. The hens were fed an antibiotic-free mash feed during this research. The diets were formulated in the feed mill located at the University of Georgia's Poultry Research Center with the following nutritional specifications: metabolizable energy: 1.26 MJ/hen/day, crude protein: 16.70 g/day, calcium: 4 g/day, and digestible phosphorus: 0.40 g/day. Husbandry and management followed the Hy-Line W-36 commercial layers management guidelines [29].

2.3. Experimental Design and Treatment

The temperature, lighting system, and ventilation rates in each room were controlled using the Chore-Tronics Model 8 controller. The light duration was maintained according to the Hy-line management guidelines, with the lowest light period being at 27 WOA (15.5 h) and the highest at 29 to 30 WOA (16 h) during the laying hen peaking phase (the hen's life cycle age from 17 to 37 WOA). In addition, the light intensity in all rooms was kept at 12 lux (11.9 ± 1.8 lux) before starting the treatment to decrease the prevalence of pecking

behavior (the aggressive behavior of hens when one hen pecks at another) among laying hens previously observed in pullet rearing [30].

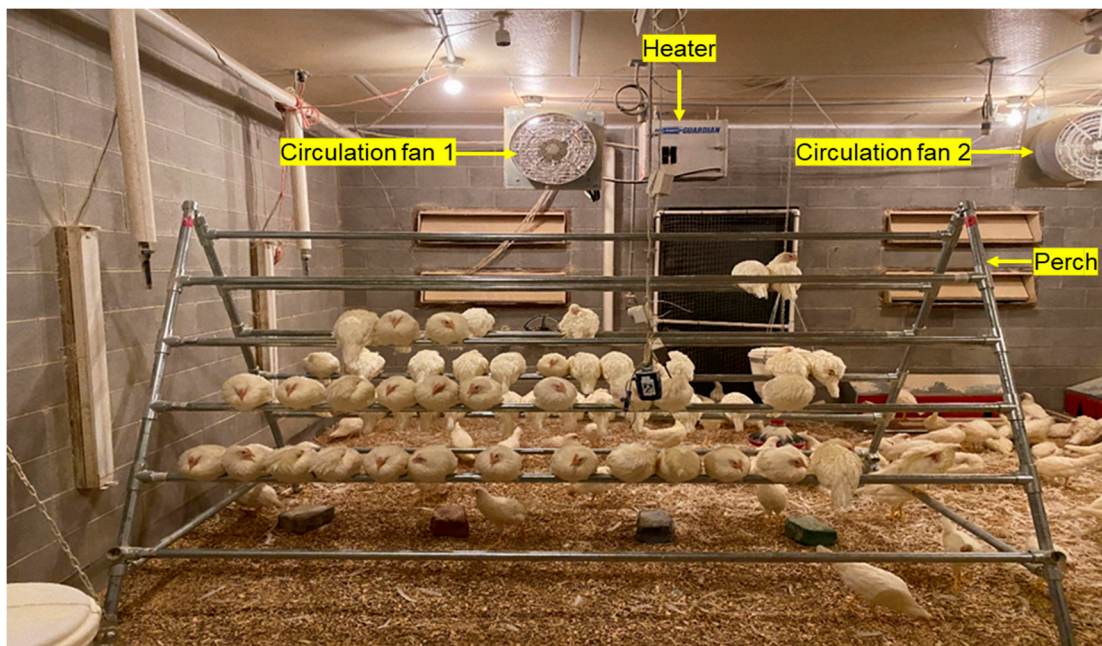


Figure 2. Experimental cage-free room used for this research.

The cage-free housing system consists of equipment attached to the ceiling, such as a heater, fan, feeder, and perches, that can block light and cause dark areas. This study found that the heater and circulating fan cause dark areas (low light intensity places compared to other places within the room). That is why we introduced additional light as treatment in those places in each room to maintain uniformity of light intensity. Since the mislaid egg counts varied significantly between the rooms before the treatment started (Figure 3), this study uses the before (without the addition of additional lighting in darker areas) and after treatment (addition of additional lighting in darker areas for uniform room lighting) concept. Data collection was conducted for 4 weeks (the first 2 weeks before treatment and the following 2 weeks after treatment) in each room.

2.4. Data Collection and Calculations

2.4.1. Floor Eggs

Eggs in each room were manually collected daily from 27 to 30 WOA. The percentage of eggs laid on the floor or mislaid eggs each week, from week 27 to 30 WOA, was determined using the mathematical formula in Equation (1). Similarly, percentages of mislaid eggs before and after treatment and mislaid eggs reduction changed over time and were calculated by the formulas given below:

$$\text{Weekly mislaid egg rate (\%)} = \frac{\text{Mislaid eggs in a week}}{\text{Total eggs in a week}} \times 100 \quad (1)$$

$$\text{Before treatment mislaid egg rate (\%)} = \frac{\text{Mislaid eggs before treatment}}{\text{Total eggs before treatment}} \times 100 \quad (2)$$

$$\text{After treatment mislaid egg rate (\%)} = \frac{\text{Mislaid eggs after treatment}}{\text{Total eggs after treatment}} \times 10 \quad (3)$$

$$\text{Mislaid eggs reduction rate (\%)} = \frac{(\text{Before treatment} - \text{After treatment}) \text{ mislaid eggs}}{\text{Before treatment mislaid eggs}} \times 100 \quad (4)$$

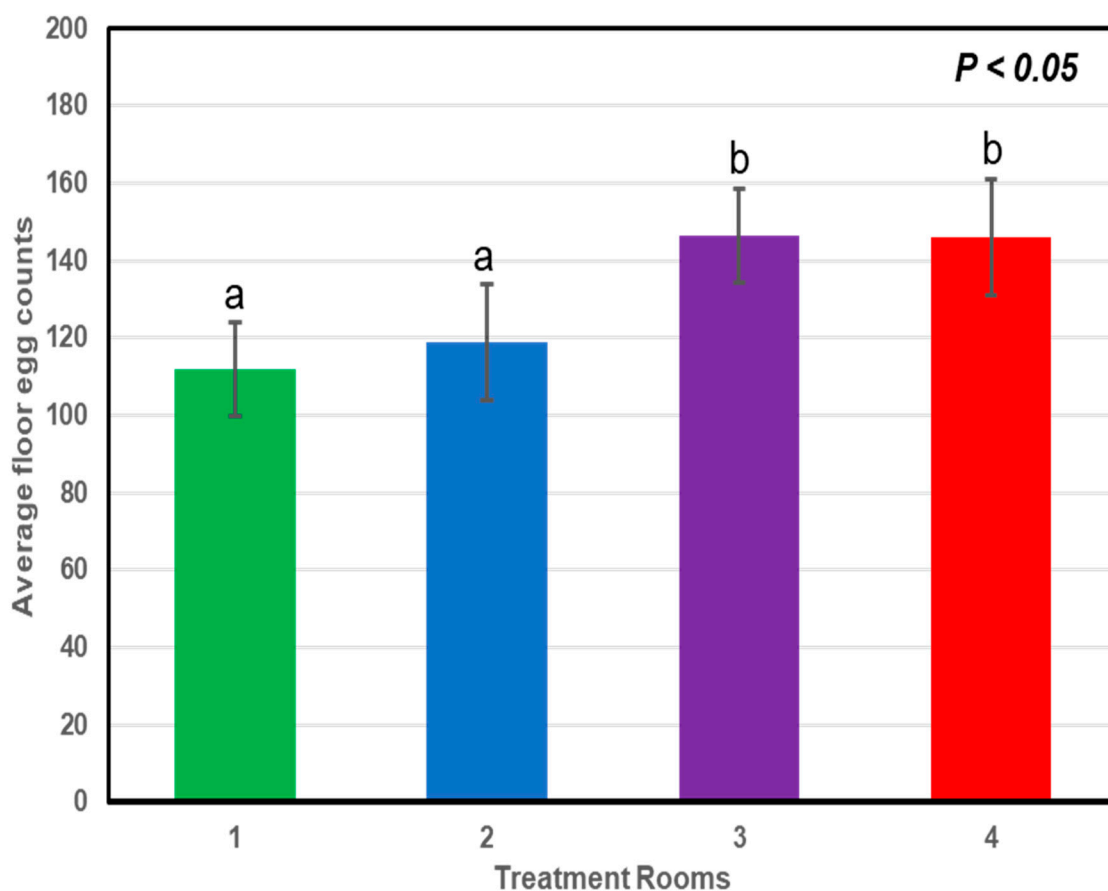


Figure 3. Floor egg counts observed before treatment in experimental cage-free rooms. The letters that differ are statistically significant at $p < 0.05$ ($n = 4$). Error bar represents the standard deviation.

2.4.2. Nest Eggs

Nest eggs were collected manually every day from each room and analyzed weekly. In addition, weekly nest egg, before and after treatment eggs number, and percentage increase in nest eggs were calculated by the formula given below:

$$\text{Weekly nest egg rate (\%)} = \frac{\text{Nest eggs in a week}}{\text{Total eggs in a week}} \times 100 \tag{5}$$

$$\text{Before treatment nest egg rate (\%)} = \frac{\text{Nest eggs before treatment}}{\text{Total eggs before treatment}} \times 100 \tag{6}$$

$$\text{After treatment nest egg rate (\%)} = \frac{\text{Number of nest eggs after treatment}}{\text{Number of eggs after treatment}} \times 100 \tag{7}$$

$$\text{Nest egg increment rate (\%)} = \frac{\text{Before treatment nest eggs} - \text{After treatment nest eggs}}{\text{Before treatment nest eggs}} \times 100 \tag{8}$$

2.4.3. Floor Egg-Laying Behavior Monitoring

Floor egg-laying behavior is the primary cause of mislaid eggs. To better understand this behavior, a study was conducted where 6 focal hens performing FELB were randomly selected from each room. These focal hens were given lightweight harnesses (8.4 g; made by our researcher) three days before the study began to make them accustomed to wearing them. Each harness was labeled 1–6 to help identify the individual hens (Figure 4). The

FELB of these focal hens was recorded 24 h a day from 27 to 30 weeks of age using six cameras attached to the ceiling of each room to capture an overview of the entire space. After the research was completed, videos recorded during daylight hours (16 h, from 5 a.m. to 9 p.m.) were analyzed in detail to differentiate between focal hens that were laying eggs on the floor (recorded as “0”) and those that were inside the nest boxes or not performing FELB (recorded as “1”). A duration of 5 min was selected to conduct this study, as previous research had indicated that focal hens typically spend at least 5 min during nest-laying behavior [13]. Therefore, if the focal hens remained inside the nest boxes or were not seen performing FELB for more than 5 min, they were recorded as “1”; otherwise, they were recorded as “0”. This distinction was made based on close observation of the recorded videos taken daily; however, one day of the video was randomly selected each week for analysis. Similarly, the video recordings were observed closely to determine the total time spent by each focal hen performing FELB on the floor.



Figure 4. Focal hens numbered from 1 to 6 to monitor floor egg-laying behavior.

2.5. Statistical Analysis

This study used four rooms as the experimental unit or replicates to track floor eggs and six individual focal hens for FELB observations. Additional lighting was used to ensure consistent light intensity throughout the rooms before and after treatment. The response was a reduction of mislaid eggs and FELB. This study included data from two weeks before and two weeks after treatment, which were analyzed using paired t-tests at a significance level of 0.05 in R Studio 4.2.1. We also used logistic regression for individual focal hen FELB data, regardless of whether they performed FELB or not.

3. Results and Discussion

3.1. Light Intensity

This study found that in areas of the poultry house where shadows fell, the light intensity was significantly lower than in other areas. This low light intensity was a major reason for increased mislaid eggs. A commercial study of aviary housing revealed that increasing the light intensity from 5 lux to 20–50 lux beneath the housing reduced mislaid eggs by up to 80% in the areas where the light was installed [25]. In this study, the low light intensity was measured to be 8.56 ± 1.29 lux at places where the shadow of different equipment attached to the ceiling falls. Therefore, additional lighting was introduced above the shadowed areas to address this issue (Figure 5), resulting in a uniform light intensity of 12.7 ± 0.2 lux throughout the room (Figure 6). Therefore, it is important to maintain a

uniform light intensity in poultry housing to prevent hens from laying eggs in undesirable locations.

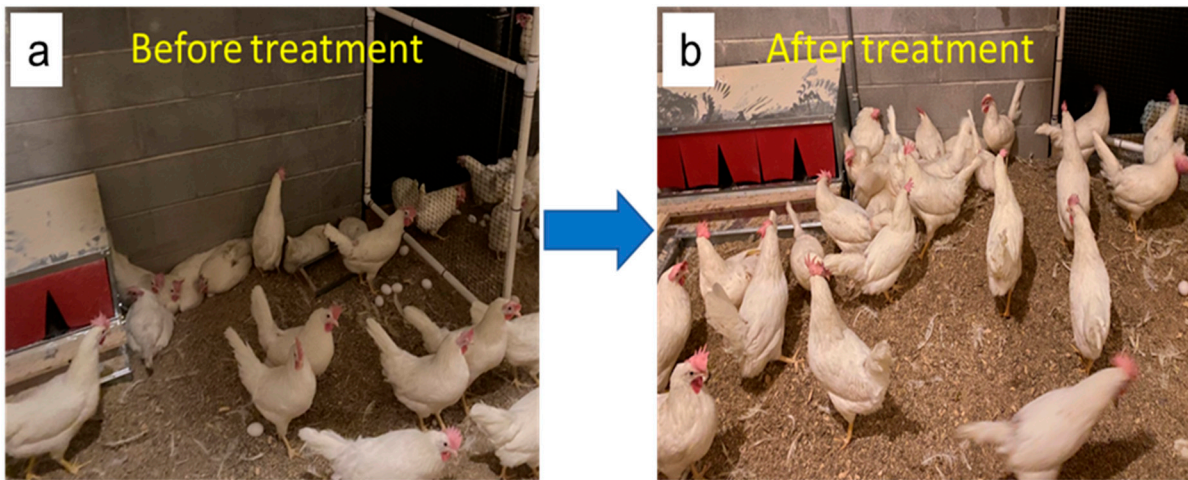


Figure 5. Lighting environment (a) before treatment (increasement of light) and (b) after treatment.

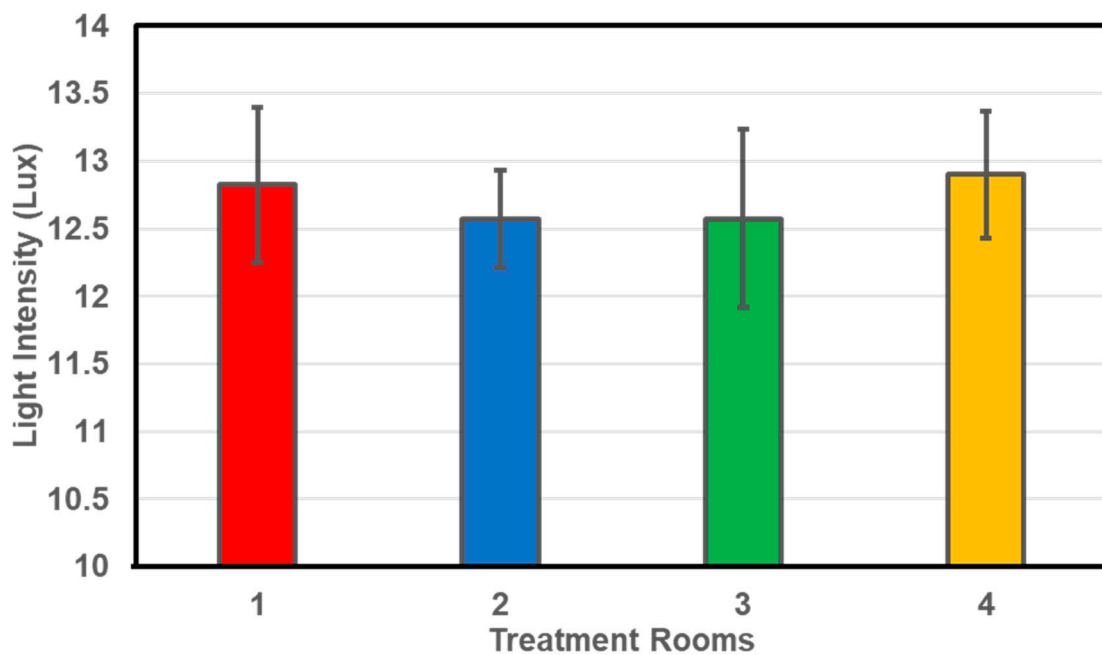


Figure 6. Light intensity inside different experimental rooms during the study.

3.2. Floor Egg Reduction

The results of this study show that the introduction of light treatment significantly affected the percentage of mislaid eggs compared to before treatments ($p < 0.05$; Figure 7). Before the treatment, the average percentage of mislaid eggs was 82.7%. After the treatment, the average percentage dropped significantly to 68.3%. Interestingly, mislaid eggs were highest in floor-raised compared to the aviary cage-free housing systems (0.2 to 10% mislaid eggs) [12]. In addition, the percentage of mislaid eggs was higher by 31.6–59.6% [13] and lower by 88% [31] than in previous research. The higher percentage of mislaid eggs could be due to housing type [16], environment [31], social interaction [32], litter depth [25], stocking density [33], genetics [34], or nest boxes occupied by dominant hens [35].

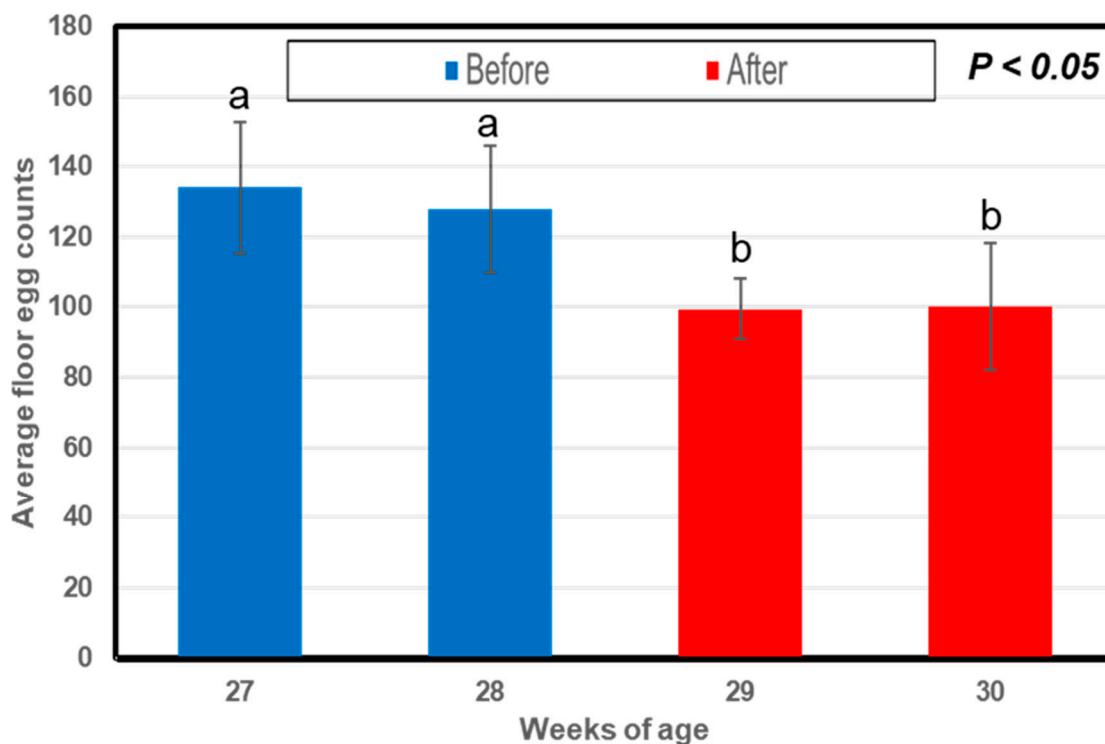


Figure 7. Average floor egg counts before and after treatment every week in a cage-free hen house (170 hens). The letters that differ are statistically significant at $p < 0.05$. The error bar represents the standard deviation.

The overall reduction in mislaid eggs due to the lighting intensity adjustment was 23.8%. Similarly, the data presented in Figure 8 for daily floor eggs indicate a significant decrease in the floor eggs after increasing of light intensity in darker areas. The floor egg reduction attributed to the increased intensity of the light in those places because hens tend to lay eggs in darker areas naturally [26]. Therefore optimizing the light intensity and uniformity is critical for managing mislaid eggs in the CF houses.

Mislaid eggs are challenging for CF egg production systems [36], so egg producers and researchers are desperate for practical solutions to prevent economic losses associated with mislaid eggs. To combat the issue of mislaid eggs, it is necessary to adopt integrated mitigation methods that address various factors, including behavioral, hormonal, environmental, and managerial aspects. Mislaid eggs are higher in cage-free floor-raised housing than in aviary housing, indicating that alternative approaches need to be explored. In this regard, litter substrate depth and types also reduce mislaid eggs, as highlighted in a recent study [25]. Therefore, effective management strategies are necessary to reduce the occurrence of floor eggs and maintain the safety and quality of egg products. Further research is also needed in this field to identify new and effective ways to mitigate the problem of mislaid eggs.

3.3. Eggs Laid in Nesting Boxes

The effective approach to increasing nest eggs in CF hen houses or breeder houses is to provide sufficient nest boxes. However, other factors such as lighting and litter management can also influence nest egg counts. This study observed that the percentage of eggs laid in the nest boxes increased from 17.3% to 31.7% after the introduction of the additional lights in darker area (Figure 9). Similarly, the data presented in Figure 10 for daily nest eggs indicate a significant increase of nest boxes eggs after treatment compared to before treatment. Overall, the percentage of nest eggs increased by 69.4%. The increase in nest eggs could be because of the hen's instinct to seek out enclosed spaces for laying their eggs, providing a sense of privacy and security.

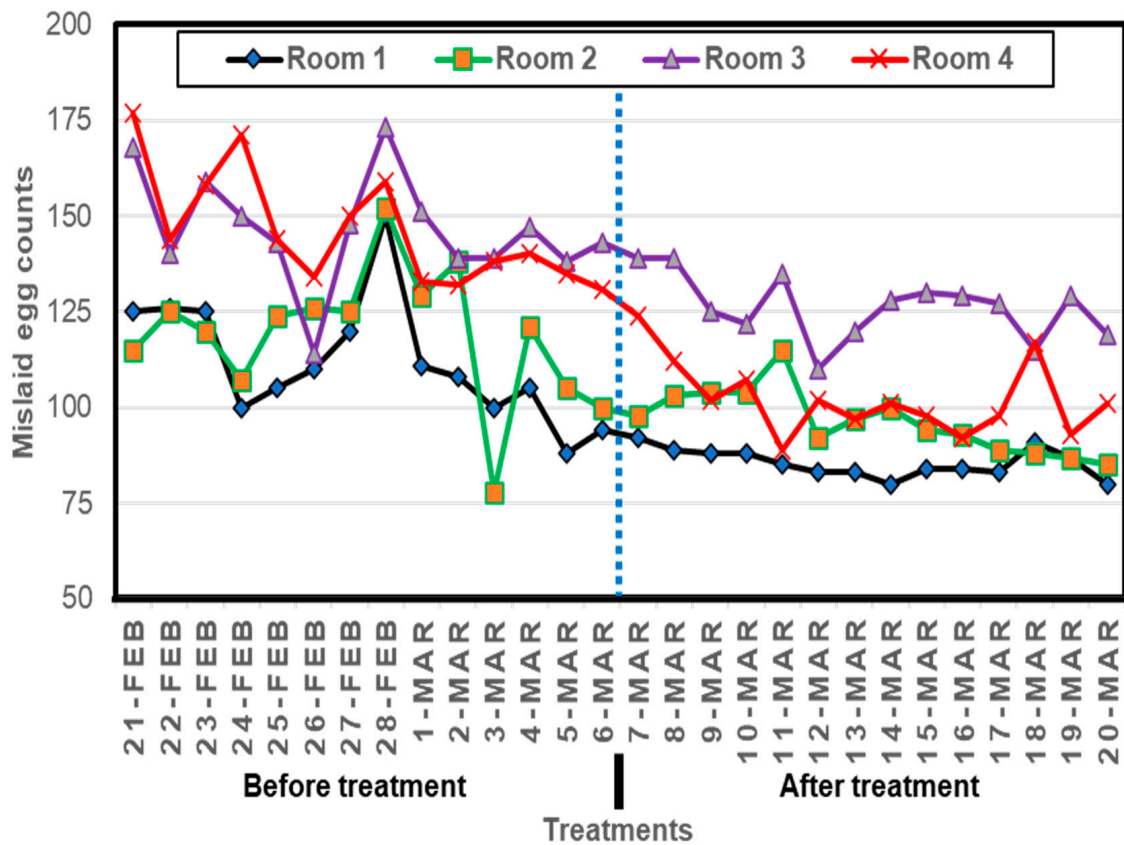


Figure 8. Comparison of daily mislaid egg numbers before and after treatment (i.e., lighting management).

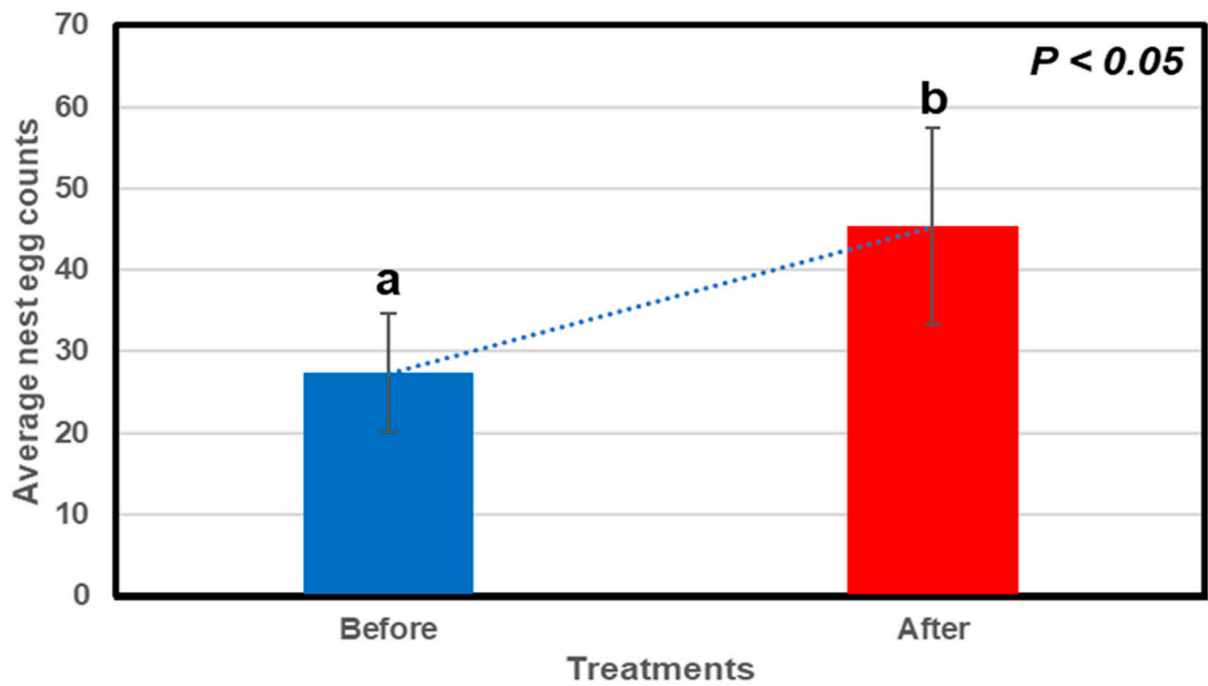


Figure 9. Average nest egg count before and after increasing light intensity. The letters that differ are statistically significant at $p < 0.05$. The error bar represents the standard deviation.

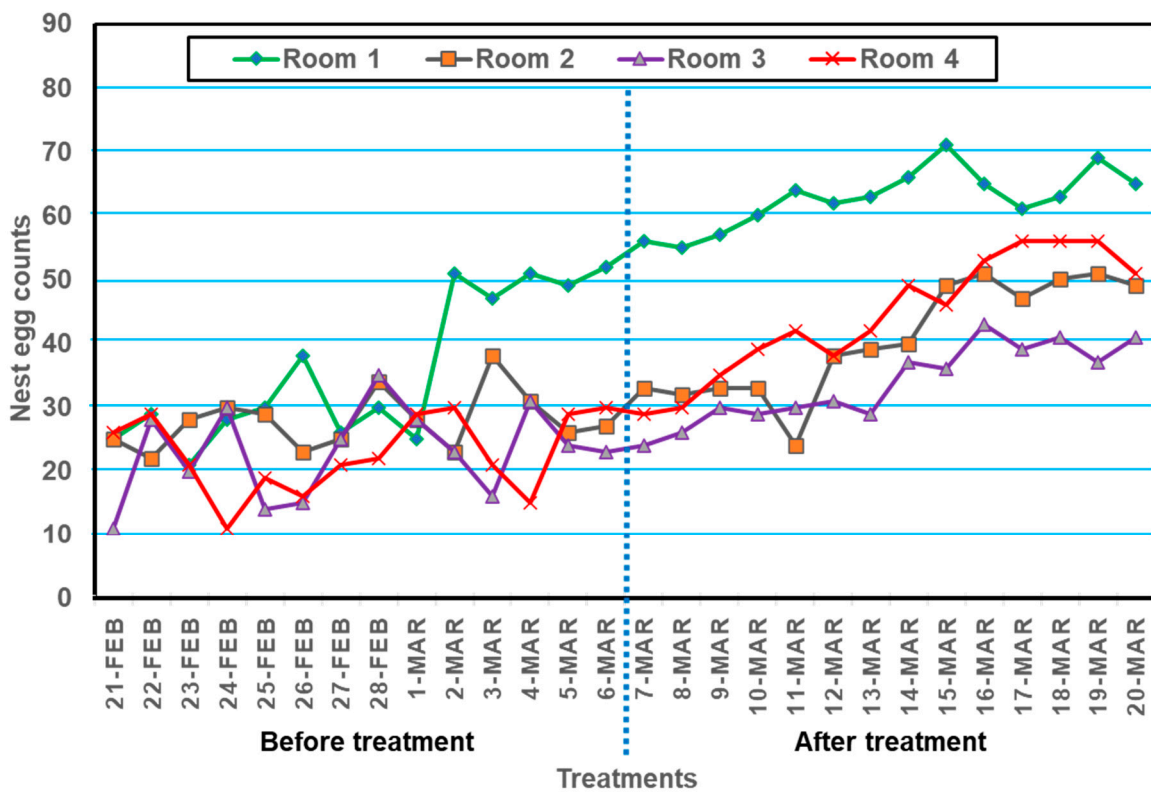


Figure 10. Comparison of daily nest egg numbers before and after treatment.

Providing nest boxes early can help reduce floor egg incidence [14]. However, in this study, nest boxes were provided at 14 WOA, but we still found a higher prevalence of floor eggs instead of nest eggs. Similarly, by providing adequate nesting space, hens are more likely to lay their eggs in boxes [37–39], which helps prevent damage to the eggs and reduces the risk of contamination, ultimately improving the quality of the eggs [40,41]. However, it is important to note that the design and placement of the nest boxes can impact their effectiveness [42,43]. The boxes should be appropriately sized for the breed of hen and be positioned in an easily accessible area that offers a sense of privacy and security. Regular cleaning and disinfection of the nest boxes are also important for maintaining the health and productivity of the flock [44–47]. In addition, taking measures such as cleaning and drying the boxes, using comfortable substrates [42,48], and creating a dimly lit environment can enhance their attractiveness [49,50]. Overall, providing nest boxes is a vital management strategy for reducing the prevalence of floor eggs in poultry housing.

3.4. Floor Egg Laying Behavior Monitoring

This research observed focal hens and found that some changed their egg-laying behavior from laying eggs on the floor to nesting. The result was a significant difference between focal hens that chose to lay eggs in designated nesting areas versus those that continued to lay eggs on the floor ($p < 0.01$). Approximately one-third of the hens preferred to lay their eggs in designated nesting areas, while the others tended to stick to one location or frequently change locations to find a safer area. The choice of egg-laying location could be influenced by the dominant hens in the flock, sometimes leading to aggression and pecking behavior among the hens [51]. When multiple hens lay eggs on the floor together, piling behavior concerns animal welfare [33,52]. The number of hens laying eggs on the floor significantly differed when they were in a group versus laying eggs individually ($p < 0.01$), which is likely due to piling behavior. To address piling and pecking behavior, different strategies should be integrated to control FELB. Investigating why hens choose

certain locations on the floor to lay their eggs could help identify the underlying causes of FELB in those areas.

3.5. Egg Laying Duration

According to this study, the time spent by individual focal hens laying eggs on the floor varied significantly ($p < 0.05$; Figure 11). This study recorded the time taken by 24 labeled birds to lay their eggs and found that it ranged from a minimum of 12.42 min (0.2 h) to a maximum of 156.19 min (2.6 h) on the floor. Compared to previous research [13], this study found that focal hens took longer to lay eggs on the floor than in their nest boxes. This prolonged duration may be due to disturbances caused by other hens during the laying period. This study found that out of 24 focal hens, 8 hens performed FELB to lay eggs in a single attempt, another 8 focal hens in the second attempt, 4 focal hens in a third attempt, and the remaining 4 hens in the fourth or more than fourth attempt. The average time taken for a first, second, third, and fourth or more than fourth attempt was 21.30 ± 9.39 , 71.45 ± 40.36 , 97.34 ± 40.08 , and 108.03 ± 69.20 min, respectively. The higher the number of attempts, the longer it took to perform FELB and lay eggs on the floor.

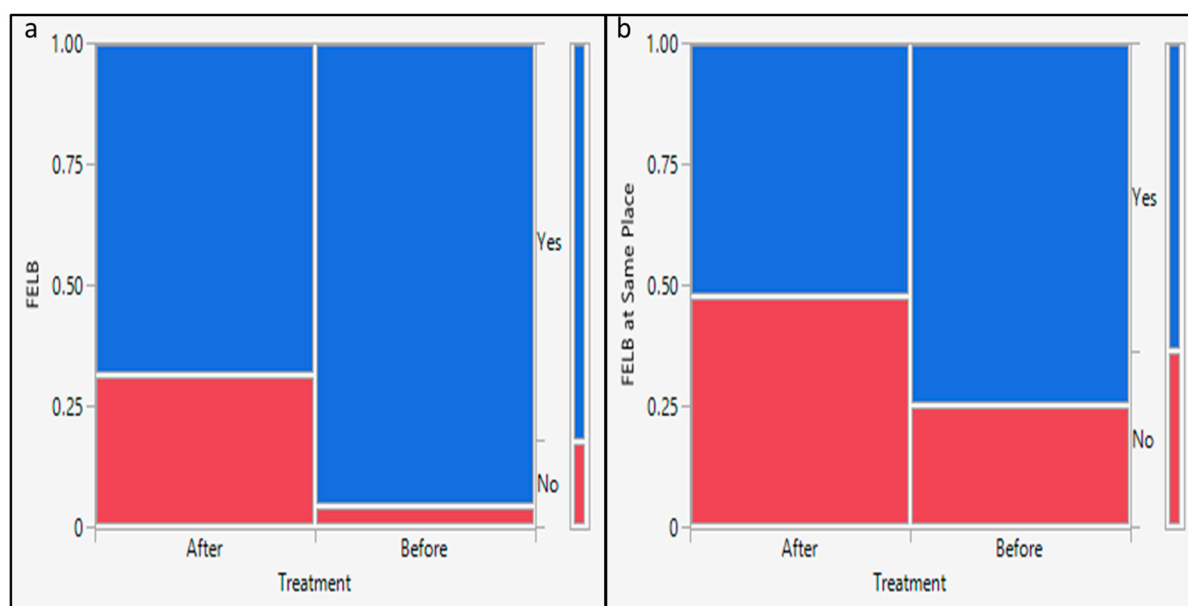


Figure 11. Mosaic plot showing the relationship between (a) FELB categorized as “Yes” for hens engaging in FELB or “No” for hens not involved in FELB and (b) FELB at the same location categorized as “Yes” for hens performing FELB in the expected location or “No” for hens performing FELB in a different location. FELB: floor egg-laying behavior.

Overall, this study highlights the significant variation in the time for focal hens to lay eggs on the floor and the negative impact of disturbances caused by other hens. The findings also suggest that FELB is not always quick or efficient for hens, especially when laying eggs on the floor. By shedding light on these factors, this study provides valuable insights into the behavior and welfare of egg-laying hens in commercial settings. Nonetheless, limitations exist within this study. For instance, there were challenges in consistently locating specific focal birds, which could be time-consuming. Furthermore, due to the similarity in appearance among the hens, it was not possible to track each one individually.

4. Conclusions

In conclusion, this study investigated the effect of lighting management on floor eggs in the cage-free production systems. Increasing lighting intensity in shadowed areas resulted in a substantial reduction of mislaid eggs and floor egg laying behavior (FELB) in laying hens. The introduction of additional lighting led to a remarkable 23.8% decrease

in floor-laid eggs. Additionally, our research investigated the floor egg-laying duration, ranging from 21.3 to 108.03 min for laying an egg on the floor. To enhance the welfare and productivity of laying hens, future studies should prioritize efforts to mitigate FELB, which are identified as root cause of mislaid eggs.

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Institutional Review Board Statement: Four identical experimental rooms were used in this research, and all procedures were approved by the Institutional Animal Care and Use Committee (IACUC) prior to the start of the study (AUF#: A2020 08-014-A1, approved on 5 October 2020).

Data Availability Statement: Data will be available per reasonable request.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. USDA. USDA Graded Cage-Free Eggs: All They're Cracked Up to Be. Available online: <https://www.usda.gov/media/blog/2016/09/13/usda-graded-cage-free-eggs-all-theyre-cracked-be> (accessed on 11 May 2023).
2. Kollenda, E.; Baldock, D.; Hiller, N.; Lorant, A. *Transitioning towards Cage-Free Farming in the EU: Assessment of Environmental and Socio-Economic Impacts of Increased Animal Welfare Standards*; Institute for European Environmental Policy: Brussels, Belgium; London, UK, 2020; pp. 1–65.
3. UEP. Retailers, Restaurants Continue Cage-Free Commitments. *United Egg Producers*. 2022. Available online: <https://unitedegg.com/retailers-restaurants-continue-cage-free-commitments/> (accessed on 5 July 2023).
4. Englmaierova, M.; Tůmová, E.; Charvátová, V.; Skřivan, M. Effects of Laying Hens Housing System on Laying Performance, Egg Quality Characteristics, and Egg Microbial Contamination. *Czech J. Anim. Sci.* **2014**, *59*, 345–352. [[CrossRef](#)]
5. Bist, R.B.; Yang, X.; Subedi, S.; Chai, L. Mislaid Behavior Detection in Cage-Free Hens with Deep Learning Technologies. *Poult. Sci.* **2023**, *102*, 102729. [[CrossRef](#)]
6. Appleby, M.C. Factors Affecting Floor Laying by Domestic Hens: A Review. *World's Poult. Sci. J.* **1984**, *40*, 241–249. [[CrossRef](#)]
7. Parisi, M.; Northcutt, J.; Smith, D.; Steinberg, E.; Dawson, P. Microbiological Contamination of Shell Eggs Produced in Conventional and Free-Range Housing Systems. *Food Control* **2015**, *47*, 161–165. [[CrossRef](#)]
8. Garcia, J.S.; Jones, D.R.; Gast, R.K.; Robison, C.I.; Regmi, P.; Karcher, D.M. Influence of Forage Substrates on Environmental and Egg Microbiology in Cage-Free Aviary Housing. *J. Appl. Poult. Res.* **2022**, *31*, 100225. [[CrossRef](#)]
9. Guard-Petter, J. The Chicken, the Egg and *Salmonella enteritidis*. *Environ. Microbiol.* **2001**, *3*, 421–430. [[CrossRef](#)] [[PubMed](#)]
10. Mench, J.; Swanson, J.; Arnot, C. The Coalition for Sustainable Egg Supply: A Unique Public–Private Partnership for Conducting Research on the Sustainability of Animal Housing Systems Using a Multistakeholder Approach. *J. Anim. Sci.* **2016**, *94*, 1296–1308. [[CrossRef](#)]
11. FDA Egg Safety: What You Need to Know. 2022. Available online: <https://www.fda.gov/media/82227/download> (accessed on 18 May 2023).
12. Vroegindewij, B.A.; Blaauw, S.K.; Ijsselmuiden, J.M.; van Henten, E.J. Evaluation of the Performance of PoultryBot, an Autonomous Mobile Robotic Platform for Poultry Houses. *Biosyst. Eng.* **2018**, *174*, 295–315. [[CrossRef](#)]
13. Li, G.; Hui, X.; Zhao, Y.; Zhai, W.; Purswell, J.L.; Porter, Z.; Poudel, S.; Jia, L.; Zhang, B.; Chesser, G.D. Effects of Ground Robot Manipulation on Hen Floor Egg Reduction, Production Performance, Stress Response, Bone Quality, and Behavior. *PLoS ONE* **2022**, *17*, e0267568. [[CrossRef](#)]
14. Cox, W. The Problem of Floor Eggs. *Canadian Poultry*. 2011. Available online: https://canadianpoultry.ca/wp-content/uploads/2020/04/breeder_floor_eggs.pdf (accessed on 1 March 2023).
15. Hunniford, M.E.; Mason, G.J.; Widowski, T.M. Laying Hens' Preferences for Nest Surface Type Are Affected by Enclosure. *Appl. Anim. Behav. Sci.* **2018**, *201*, 7–14. [[CrossRef](#)]
16. Gunnarsson, S. Effect of Rearing Factors on the Prevalence of Floor Eggs, Cloacal Cannibalism and Feather Pecking in Commercial Flocks of Loose Housed Laying Hens. *Br. Poult. Sci.* **1999**, *40*, 12–18. [[CrossRef](#)] [[PubMed](#)]
17. Li, G.; Xu, Y.; Zhao, Y.; Du, Q.; Huang, Y. Evaluating Convolutional Neural Networks for Cage-Free Floor Egg Detection. *Sensors* **2020**, *20*, 332. [[CrossRef](#)] [[PubMed](#)]

18. Santos, R.C.; Lopes, A.L.; Sanches, A.C.; Gomes, E.P.; da Silva, E.A.; da Silva, J.L. Intelligent Automated Monitoring Integrated with Animal Production Facilities. *Eng. Agrícola* **2023**, *43*, e20220225. [[CrossRef](#)]
19. EFSA Scientific Opinion on the Public Health Risks of Table Eggs Due to Deterioration and Development of Pathogens. *EFSA J.* **2014**, *12*, 3782. [[CrossRef](#)]
20. USDA Inspection & Mission Training. Food Safety and Inspection Service. Available online: <http://www.fsis.usda.gov/inspection/inspection-training-videos/inspection-mission-training> (accessed on 22 October 2023).
21. USDA. *Sanitation Requirements*; Food Safety and Inspection Service—U.S. Department of Agriculture: Washington, DC, USA, 2021.
22. Yang, X.; Bist, R.B.; Subedi, S.; Chai, L. A Computer Vision-Based Automatic System for Egg Grading and Defect Detection. *Animals* **2023**, *13*, 2354. [[CrossRef](#)] [[PubMed](#)]
23. Li, G.; Chesser, G.D.; Huang, Y.; Zhao, Y.; Purswell, J.L. Development and Optimization of a Deep-Learning-Based Egg-Collecting Robot. *Trans. ASABE* **2021**, *64*, 1659–1669. [[CrossRef](#)]
24. Subedi, S.; Bist, R.; Yang, X.; Chai, L. Tracking Floor Eggs with Machine Vision in Cage-Free Hen Houses. *Poult. Sci.* **2023**, *102*, 102637. [[CrossRef](#)]
25. Chai, L.; Dunkley, C.; Ritz, C. Mislaid Egg Management in Cage-Free Hen Houses. University of Georgia Extension Publication. 2023. Available online: https://secure.caes.uga.edu/extension/publications/files/pdf/C%201254_1.PDF (accessed on 22 June 2023).
26. Yalcinalp, M. Broiler Breeder Management to Minimize Floor Egg Production. Cobb-Vantress. 2019. Available online: <https://www.cobb-vantress.com/assets/7681ca01bb/Mert-Yalcinalp-Floor-Eggs.pdf> (accessed on 12 July 2023).
27. Abrahamsson, P.; Tauson, R. Performance and Egg Quality of Laying Hens in an Aviary System. *J. Appl. Poult. Res.* **1998**, *7*, 225–232. [[CrossRef](#)]
28. UEP CF-UEP-Guidelines. Available online: https://uepcertified.com/wp-content/uploads/2021/08/CF-UEP-Guidelines_17-3.pdf (accessed on 18 August 2022).
29. Hy-Line 36 COM ENG.Pdf. Available online: <https://www.hyline.com/filesimages/Hy-Line-Products/Hy-Line-Product-PDFs/W-36/36%20COM%20ENG.pdf> (accessed on 10 September 2022).
30. Bist, R.B.; Subedi, S.; Yang, X.; Chai, L. Effective Strategies for Mitigating Feather Pecking and Cannibalism in Cage-Free W-36 Pullets. *Poultry* **2023**, *2*, 281–291. [[CrossRef](#)]
31. Hughes, B. Preference Decisions of Domestic Hens for Wire or Litter Floors. *Appl. Anim. Ethol.* **1976**, *2*, 155–165. [[CrossRef](#)]
32. Sherwin, C.; Nicol, C. Factors Influencing Floor-Laying by Hens in Modified Cages. *Appl. Anim. Behav. Sci.* **1993**, *36*, 211–222. [[CrossRef](#)]
33. Bist, R.B.; Subedi, S.; Yang, X.; Chai, L. A Novel YOLOv6 Object Detector for Monitoring Piling Behavior of Cage-Free Laying Hens. *AgriEngineering* **2023**, *5*, 905–923. [[CrossRef](#)]
34. McGibbon, W. Floor Laying—A Heritable and Environmentally Influenced Trait of the Domestic Fowl. *Poult. Sci.* **1976**, *55*, 765–771. [[CrossRef](#)]
35. Cordiner, L.; Savory, C. Use of Perches and Nestboxes by Laying Hens in Relation to Social Status, Based on Examination of Consistency of Ranking Orders and Frequency of Interaction. *Appl. Anim. Behav. Sci.* **2001**, *71*, 305–317. [[CrossRef](#)] [[PubMed](#)]
36. Holt, P.; Davies, R.; Dewulf, J.; Gast, R.; Huwe, J.; Jones, D.; Waltman, D.; Willian, K. The Impact of Different Housing Systems on Egg Safety and Quality. *Poult. Sci.* **2011**, *90*, 251–262. [[CrossRef](#)] [[PubMed](#)]
37. Appleby, M.C.; McRae, H.E. The Individual Nest Box as a Super-Stimulus for Domestic Hens. *Appl. Anim. Behav. Sci.* **1986**, *15*, 169–176. [[CrossRef](#)]
38. Cooper, J.J.; Appleby, M.C. Nesting Behaviour of Hens: Effects of Experience on Motivation. *Appl. Anim. Behav. Sci.* **1995**, *42*, 283–295. [[CrossRef](#)]
39. Cooper, J.J.; Appleby, M.C. Demand for Nest Boxes in Laying Hens. *Behav. Process.* **1996**, *36*, 171–182. [[CrossRef](#)]
40. Berrang, M.; Frank, J.; Buhr, R.; Bailey, J.; Cox, N.; Mauldin, J. Microbiology of Sanitized Broiler Hatching Eggs through the Egg Production Period. *J. Appl. Poult. Res.* **1997**, *6*, 298–305. [[CrossRef](#)]
41. De Reu, K. *Bacteriological Contamination and Infection of Shell Eggs in the Production Chain*; Ghent University: Ghent, Belgium, 2006; ISBN 90-5989-124-4.
42. Wall, H.; Tauson, R.; Elwinger, K. Effect of Nest Design, Passages, and Hybrid on Use of Nest and Production Performance of Layers in Furnished Cages. *Poult. Sci.* **2002**, *81*, 333–339. [[CrossRef](#)]
43. Hunniford, M.E.; Widowski, T.M. Nest Alternatives: Adding a Wire Partition to the Scratch Area Affects Nest Use and Nesting Behaviour of Laying Hens in Furnished Cages. *Appl. Anim. Behav. Sci.* **2017**, *186*, 29–34. [[CrossRef](#)]
44. Rendell, W.B.; Verbeek, N.A. Old Nest Material in Nest Boxes of Tree Swallows: Effects on Nest-Site Choice and Nest Building. *Auk* **1996**, *113*, 319–328. [[CrossRef](#)]
45. Utsey, F.M.; Hepp, G.R. Frequency of Nest Box Maintenance: Effects on Wood Duck Nesting in South Carolina. *J. Wildl. Manag.* **1997**, *61*, 801–807. [[CrossRef](#)]
46. Podofilini, S.; Cecere, J.G.; Griggio, M.; Curcio, A.; De Capua, E.L.; Fulco, E.; Pirrello, S.; Saino, N.; Serra, L.; Visceglia, M. Home, Dirty Home: Effect of Old Nest Material on Nest-Site Selection and Breeding Performance in a Cavity-Nesting Raptor. *Curr. Zool.* **2018**, *64*, 693–702. [[CrossRef](#)] [[PubMed](#)]
47. Dulisz, B.; Stawicka, A.M.; Knozowski, P.; Diserens, T.A.; Nowakowski, J.J. Effectiveness of Using Nest Boxes as a Form of Bird Protection after Building Modernization. In *Biodiversity and Conservation*; Springer: Berlin, Germany, 2022; pp. 1–18.

48. Struelens, E.; Van Nuffel, A.; Tuytens, F.A.; Audoorn, L.; Vranken, E.; Zoons, J.; Berckmans, D.; Ödberg, F.; Van Dongen, S.; Sonck, B. Influence of Nest Seclusion and Nesting Material on Pre-Laying Behaviour of Laying Hens. *Appl. Anim. Behav. Sci.* **2008**, *112*, 106–119. [[CrossRef](#)]
49. Potzsch, C.; Lewis, K.; Nicol, C.; Green, L. A Cross-Sectional Study of the Prevalence of Vent Pecking in Laying Hens in Alternative Systems and Its Associations with Feather Pecking, Management and Disease. *Appl. Anim. Behav. Sci.* **2001**, *74*, 259–272. [[CrossRef](#)]
50. Struelens, E.; Tuytens, F.; Janssen, A.; Leroy, T.; Audoorn, L.; Vranken, E.; De Baere, K.; Ödberg, F.; Berckmans, D.; Zoons, J. Design of Laying Nests in Furnished Cages: Influence of Nesting Material, Nest Box Position and Seclusion. *Br. Poult. Sci.* **2005**, *46*, 9–15. [[CrossRef](#)] [[PubMed](#)]
51. Subedi, S.; Bist, R.; Yang, X.; Chai, L. Tracking Pecking Behaviors and Damages of Cage-Free Laying Hens with Machine Vision Technologies. *Computers and Electronics in Agriculture* **2023**, *204*, 107545. [[CrossRef](#)]
52. Campbell, D.L.M.; Makagon, M.M.; Swanson, J.C.; Siegford, J.M. Litter use by laying hens in a commercial aviary: Dust bathing and piling. *Poult. Sci.* **2016**, *95*, 164–175. [[CrossRef](#)]

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