Factors affecting space use by laying hens in a cage-free aviary system: effect of nest lighting at pullet housing and of curtain nest color during laying

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ABSTRACT At 17 wk of age, 1,800 Lohman brown hens were housed in 8 pens of an experimental aviary system, specifically set up for the purposes of the present study, and kept until 26 wk without or with nest lighting (lights inside the nest 1.5 h before the lighting of the installation) for training in the nest use. Then, at 27 wk, 4 combinations of nest curtains were adopted to evaluate the effects on hens' distribution, that is, nests with red (**RR**) or yellow (**YY**) curtains at all tiers; nests with red and yellow curtains at the first and second tier, respectively (**RY**); or nests with yellow and red curtains at the first and second tier, respectively (**YR**). The use of enlightened compared to dark nests at housing increased the oviposition rate ($P < 0.001$) and decreased the rate of broken ($P < 0.001$) and dirty eggs ($P < 0.05$) from 27 to 45 wk, while increasing the rate of eggs laid inside the nests ($P < 0.001$). The presence of yellow nest curtains increased the rate of hens on the floor in pens YY and YR compared to pens RR and RY (35.3 and 35.5% vs. 34.1 and 33.3%, respectively; $P = 0.05$) and the rate of floor eggs in pens YR (2.23% vs. 1.63 and 1.65% in pens RR and RY; $P < 0.05$). In pens RY, a higher rate of eggs was always found on the second tier compared to the first one with the most inhomogeneous distribution compared to pens RR, YY, and YR (+10.8 vs. +3.4, +1.9, and +4.6 percentage points of eggs laid on the second tier compared to the first one, respectively). In conclusion, nest lighting at housing trained hens to the use of nests while improving egg production in terms of quantity and quality. The use of yellow curtains on nests moved hens between the different levels of the aviary but this was not associated with an increased nest use for laying.

Key words: laying, hen distribution, aviary, nest lighting, color preference

2023 Poultry Science 102:102524
https://doi.org/10.1016/j.psj.2023.102524

INTRODUCTION

In Europe, around 44% of laying hens are kept in enriched cages (European Commission, 2022), whereas the European Resolution P9_TA(2021)0295 (European Parliament, 2021), answering the European Citizens' Initiative "End the Cage Age," asks for banning any cage system for farmed animals in the EU. Thus, the European Parliament has called for a phasing out of cages by 2027, which is consistent with the EU Green Deal and the Farm to Fork Strategy.

Alternative or cage-free systems for hens in Europe can be classified as barns (33.9% of farmed hens in Europe, 40.2% in Italy), including aviaries, under conventional indoor housing systems; and free-range (11.9% in Europe, 4.9% in Italy) and organic systems (6.2% in Europe, 5.5% in Italy) as outdoor productions (EU, 1999/74/EC; EC, 2008; Kollenda et al., 2020). Implementation of cage-free systems differs among European Countries and farming practices are not yet fully standardized, especially in the Mediterranean area countries such as Italy or Spain.

Field experience and literature have identified both strengths and weaknesses of cage-free systems for the production and welfare of hens (Hartcher and Jones, 2017; Gautron et al., 2022), which requires further insight to identify best on-farm solutions. In fact, enriched cages, intended to provide additional space and resources for satisfying hen behavioral needs, are

Received November 25, 2022.
Accepted January 17, 2023.

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relatively homogenous in structures and equipment all over farms and have a relatively low risk for diseases and parasitism under controlled conditions, besides high economic efficiency (Lay et al., 2011; Mench et al., 2011; Campbell et al., 2019). On the other hand, each cage-free housing system has characteristics that can influence the hen behavior, the space use and the discarded eggs (Ali et al., 2020; Sulimova et al., 2020) whereas a correct use of the nest and a homogenous distribution of animals in the different areas of the aviary are critical. Hens are often synchronous in their behaviors (e.g., movement, dust bathing, laying), which can lead to overcrowding in specific parts of the aviary and cause undesired behaviors such as flock piling, that is, dense clustering (overcrowding) of hens mainly along walls and in corners, which can result in smothering and high losses (Campbell et al., 2016a; Winter et al., 2021).

Lighting and its management (e.g., hours of lighting, light wavelengths, light distribution) are known to affect hen behavior as for their circadian activity, aggression, and use of spaces, besides oviposition rate and egg quality (Er et al., 2007; Parvin et al., 2014; Barros et al., 2020). As hens prefer to lay in places with a low light intensity (<1 lux; Ma et al., 2016), nests do not usually have a light inside. However, lighting inside the nests could be used to attract hens since some authors found that cavity-nesting birds showed a preference for nest boxes with light compared to dark nest boxes (Podkowa and Surmacki, 2017). Similarly, hen preferences for specific colors can be exploited to manage hens' distribution in a cage-free system and optimize nest use, as there is evidence that chickens do not like some colors (e.g., blue) while they have a preference for others (e.g., yellow; Jones et al., 2000).

Thus, the present study used an experimental aviary to test first the hypothesis that nest lighting from housing in the production farm (17 wk of pullet age) until the egg deposition rate was higher than 90% (from 26 wk of age) attract brown hens to nests, and can be a strategy for training them to correctly use nests during the subsequent laying period (28−45 wk of age). Second, the study aimed at testing whether nest curtains different colors (yellow or red) can affect hen distribution and egg-laying position in the aviary during the laying period (28−45 wk of age).

MATERIALS AND METHODS

Ethics Statement

The study was approved by the Ethical Committee for Animal Experimentation (Organismo per la Protezione del Benessere Animale, **OPBA**) of the University of Padova (project 28/2020; Prot. n. 204398). All animals were handled according to the principles stated by the EU Directive 2010/63/EU (EU, 2010) regarding the protection of animals used for scientific purposes. Research staff involved in animal handling were animal specialists (PhD or MS in Animal Science) and veterinary practitioners.

Experimental Facilities and Animals

The trial was run at the Experimental Farm "Lucio Toniolo" of the University of Padova (Legnaro, Padova, Italy). The building was equipped with a cooling system, forced ventilation, radiant heating, and controlled lighting systems. For the purposes of the present study an experimental aviary was set up in the farm, which consisted of 2 tiers, equipped with collective nests (1 nest per 60 hens) closed by red plastic curtains (6 curtains of 18 cm per nest separated by 2 cm), and a third perched level. The aviary was equipped with 2 nest belts (at the first and second tiers, respectively) on which eggs were collected by hand distinguishing between those laid in and rolled out from each nest, and those laid on the wire mesh and rolled out on the belt per each pen (Figure 1). The 2 tiers also included perches, nipple drinkers and automatic feeding, and the third level had only perches and feeders. A total of 16.2 cm perches per hen was available in the whole aviary: at the first and second tiers feeding round perches and external round perches were 4.3 and 2.2 cm per hen, respectively, and rectangular perches attached to the tiers were 4.3 cm per hen; at the third level, round perches were 5.4 cm per hen. The whole aviary system was 2.50 m wide × 19.52 m long × 2.24 m high and 2 corridors per side were available, each 1.70 m wide. Thus, free ground space was 5.90 m wide × 19.52 m long. The experimental aviary was then divided into 8 pens, each with a length of 2.44 m to obtain a suitable number of replicates.

A total of 1,800 17-wk-old Lohmann Brown-Classic hens (Lohmann Tierzucht GmbH, Cuxhaven, Germany) were delivered by an authorized truck from a commercial farm to the experimental farm. On arrival, hens were randomly allocated in the 8 pens of the aviary (225 hens per pen; 9 hens/m^2 available surface). Hens were monitored until 45 wk of age, from February to September, during which only 4 hens died (average mortality 0.22%). During this period, the minimum and maximum temperature inside the barn averaged at 20.2 ± 1.1°C and 24.4 ± 2.4°C, respectively, with average minimum and maximum relative humidity at 48.2 ± 9.8% and

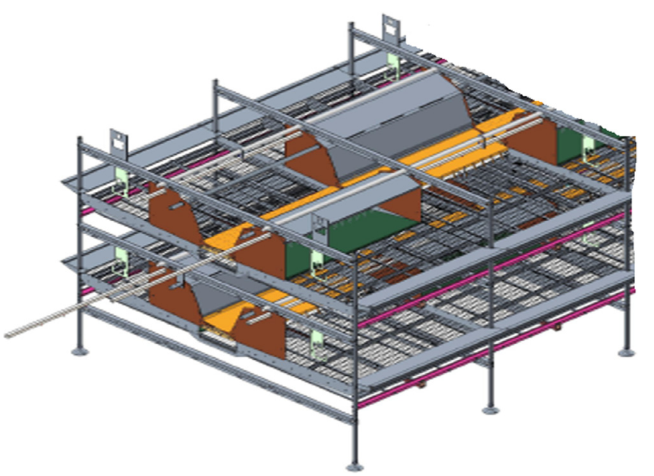

Figure 1. Drawing of the single pen of the experimental aviary used in the present study: first and second tiers.

69.7 ± 6.8%, respectively. During the whole trial, hens were fed ad libitum 4 different commercial diets formulated according to hen nutritional requirements and egg production (Lohmann Tierzucht, 2016; FEDNA et al., 2019).

At the barn level, 10 h of light were provided during the first week following the arrival of pullets, which progressively increased until 14 h of light (23 lux) after 4 wk to remain stable thereafter. For the lighting program, the sunrise was set at 7 min inside the aviary followed by 5 min in the corridors; the sunset was 10 min in the corridors followed by 17 min inside the aviary. Additionally, the nests were opened 15 min after the light of the aviary was switched on (5.30 with 14 h of light) and the nests were closed at 16.30.

Experimental Arrangement

From 17 to 26 wk of hen age, to train and attract hens toward the nests, 4 pens had the nests of both tiers opened with the inner led lights turned on at a relatively low intensity (10 lux) 1.5 h before until 1.5 h after the light of the corridors in the barn turned on; the other 4 pens had the nests closed until turning on the lights of the corridors and without any light switched on inside. From 27 wk of age onward, in all pens, nest lights were always switched off and the entrance of the nests opened 15 min after the lights of the corridors switched on.

To evaluate the preference of hens for different colors of nest curtains, when hens reached 27 wk of age, the curtains of the nests within the pens were changed to have the following 4 experimental groups randomly distributed within the nest lighting treatments: *i*) 2 pens (one per nest light treatment) with the original red curtains in the nests of both tiers (**RR**); *ii*) 2 pens (one per nest light treatment) with the original red curtains on the first tier and yellow curtain on the second tier (**RY**); *iii*) 2 pens (one per nest light treatment) with yellow curtains in nests of both tiers (**YY**); *iv*) 2 pens (one per nest light treatment) with yellow curtains on the first tier and the original red curtains on the second tier (**YR**). After 10 wk (hens aged 37 wk), the original set up, that is, all nests with red curtains, was brought back.

Recordings

From 17 to 26 wk of age, the collection of data was addressed to evaluate the distribution of animals in the aviary as published by Pillan et al. (2020). From 28 to 45 wk of age, the numbers of total eggs, dirty and broken eggs were counted 3 d/wk. Eggs were visually classified distinguishing the damages in eggs presenting linear cracks, hole cracks, star cracks, and empty eggshell (Simons et al., 2017). The egg production over the week was calculated at the pen level as: Oviposition rate (% present hen) = total eggs in 3 recordings/total number of housed hens at the 3 recordings × 100.

The laying location of the eggs was recorded in the single pens (Figure 1). We distinguished among eggs collected on the egg belts, separating eggs laid in the nests and eggs laid (out of the nests) on the wire mesh of the tiers and rolled on the egg belts; eggs laid and found on the wire mesh of the tiers; and eggs laid on the floor. Moreover, from 28 to 45 wk of age, the distribution of the hens in the different areas of the aviary (floor, wire mesh of the first and second tiers; feeding perches and external perches of the first and second tiers; perches of the third level; and nests) within each pen was recorded once per week at 2 observation hours (8.30 and 13.30) by direct observation of the number of hens per area. Then, the hen distribution was obtained as the ratio between the number of animals detected in every part of the aviary per pen and the total number of hens per pen.

Finally, from 28 to 45 wk of age, about every 15 d for a total of 8 recordings, 800 eggs per sampling day (100 per pen) were scored for weight (g) using an electronic balance (precision 0.01 g; Model BC, Orma s.r.l., Milan, Italy), height and width (mm) using an electronic digital caliper (precision 0.01 mm; Maurer; Ferritalia, Padova, Italy) as well as all defects using an egg tester light (IM 35 Power Lux; Fiem, Guanzate, Como, Italy). The egg shape index (width/height ratio) and egg surface area (cm^2) ($4.68 \times$ egg weight$^{2/3}$) were calculated according to Sirri et al. (2018). The following defects were scored: line cracks, star cracks, shell-less eggs and soft-shelled eggs (with some shell egg formation but too little to give any strength), sandpaper shells (that feel like sandpaper, rough, and uneven), little and large pimples (calciferous materials on the eggshell), misshapen eggs, slab-sided eggs (eggs with a slabbed thin side) and double yolk (Simons et al., 2017).

Statistical Analysis

In order to test whether training by nest lighting during the first period (17–26 wk of age) had any effect on pen data of oviposition rates, egg defects and traits, distribution of laid eggs and hens in the aviary during the subsequent productive period, data collected from 28 to 45 wk of age (when all nests had light switched off and egg deposition rate was higher than 90%) were submitted to an analysis of variance (**ANOVA**) with a mixed model which included nest lighting as the main fixed effect. The pen, week of age, and nest curtain treatment were included in the model as random effects to account for any uncontrolled effect that might have affected those pens subjected to the same training by nest lighting.

To evaluate the preference of hens for curtains color between 28 and 37 wk of age, data related to pen distribution of laid eggs and distribution of hens in the aviary were submitted to an ANOVA using a mixed model that included the curtain color as fixed effect. The pen, week of age, and nest lighting treatments were included in the model as random effects to account for any uncontrolled effect that might have affected those pens subjected to the same color nest treatment.

The choice of analyzing separately, as described above, the main effects (nest lighting, curtain color) tested in this study was done based on 2 main reasons i) the low number of replications (pens), for which it was not possible to analyze data using the 2 experimental treatments and their interaction as the main effects; ii) the treatment "curtain color" was applied only during one period of the trial.

The PROC GLIMMIX of SAS (SAS Institute, 2013) was used for all analyses. Least square means were compared using Tukey's t test. Differences between the means with $P \leq 0.05$ were considered statistically significant.

RESULTS AND DISCUSSION

Effect of Nest Lighting at Housing

Regarding the main problems in commercial cage-free systems, literature reports that inadequate nest utilization by hens can lead to 4.4 to 9.5% of eggs laid outside of the nests (Oliveira et al., 2016; Villanueva et al., 2017) which implies an increase in labor load as well as economic losses for the farmers. Among the various environmental factors, light management is largely recognized to play a crucial role in the production and the behavior of laying hens (Jácome et al., 2014). Therefore, we hypothesized that lighting could be used to train hens to the use of the nests, which was also based on their preference for light presence.

Under the conditions of our trial, the average oviposition rate from 28 to 45 wk of age was significantly higher in hens kept in pens with nest lighting at housing compared to those without it (92.1% vs. 89.6%; $P < 0.001$). This result is consistent with the positive effect of a photo stimulation on induction of reproduction and on later egg production level, whereas the increase of the number of light hours during the following period has been found to have no effect on the egg production (Sharp, 1993; Lewis et al., 2007; Jácome et al., 2014). Under our conditions, in the aviary specifically set up in the experimental farm, lower rates of broken eggs (−1.1 percentage points; $P < 0.001$) and dirty eggs (−0.54 percentage points; $P < 0.05$) over the total of laid eggs were also recorded in the pens with nest lighting with respect to those without, while no differences were found in the occurrence of the different types of broken eggs (i.e., different types of cracks and empty egg shells) (Table 1). On the other hand, the average data we recorded for broken and dirty eggs were rather higher than what reported under field conditions because of the specific set up (height of the upper perches) of the experimental aviary and the management of the experimental conditions (absence of dust blowers). As for the egg scoring, the use of nest lighting was associated with smaller eggs, as for weight, height, and surface ($0.05 < P < 0.01$; Table 2), which is consistent with the previously found negative correlation between egg production and egg weight (Sterling et al., 2003). A higher rate of eggs with sandpaper shells ($P < 0.05$) was also recorded in pens with nest lighting compared to pens without nest lighting (Table 2) but average values were very low (0.38 and 0.13% of scored eggs, respectively).

Table 1. Effect of nest lighting at housing in an experimental aviary on egg production; rate of clean, dirty and broken eggs (% of total eggs); and rate of defects for broken eggs (average LS means values of samplings from 28 to 45 wk of age).

Variables	Nest lighting[1] Off	Nest lighting[1] On	P value	SEM
Recordings[2] (n)	72	72		
Egg production (% present hens)	89.6	92.1	<0.001	0.6
Egg quality (% of total eggs)				
Clean eggs	88.9	90.6	<0.001	1.0
Dirty eggs	6.69	6.15	<0.05	0.58
Broken eggs	4.40	3.29	<0.001	0.43
Defects (% broken eggs)				
Linear crack	34.9	34.4	0.796	2.2
Hole crack	0.34	0.71	0.138	0.20
Star crack	12.0	12.3	0.820	1.4
Empty eggshell	52.7	52.1	0.750	1.7

[1]On: from 17 to 26 wk of age, hens were kept in pens with the nests opened with the inner led lights turned on at low intensity (10 lux) 1.5 h before the light of the installation turned on (4 pens). Off: from 17 to 26 wk of age, hens were kept in pens with the nests closed until turning on the installation light and without any light inside.

[2]Number of data collected along the samplings from 28 to 45 wk of age.

The positive effect of nest lighting on the reduction of broken and dirty eggs points out to the benefits of this strategy to train hens to use the nests. This is also proven by the results about the position of eggs in the aviary. In fact, despite the rate of eggs on the floor being significantly higher (1.53% vs. 1.30% total eggs, respectively; $P < 0.05$), the magnitude of the difference may be considered as relatively unimportant from a quantitative point of view when compared to the higher rate of eggs being laid inside the nests (87.2% vs. 84.8% total laid eggs; $P < 0.001$) in pens which used nest lighting at

Table 2. Effect of nest lighting at housing in an experimental aviary on egg physical traits and defects (average LS means values of samplings from 28 to 45 wk of age).

Variables	Nest lighting[1] Off	Nest lighting[1] On	P value	SEM
Eggs (n)	3,200	3,200		
Egg physical traits				
Weight (g)	62.9	62.6	<0.01	0.2
Height (mm)	56.7	56.4	<0.05	0.2
Width (mm)	44.4	44.2	0.108	0.1
Shape index	0.78	0.78	0.890	0.00
Surface (cm^2)	74.0	73.7	<0.01	0.1
Defects (% eggs)				
All defects	1.50	1.53	0.903	0.21
Line cracks	0.34	0.34	1.000	0.12
Star cracks	0.19	0.19	1.000	0.08
Shell-less eggs and soft-shelled eggs	0.28	0.34	0.586	0.12
Little and large pimples	0.34	0.22	0.321	0.09
Sandpaper shells	0.13	0.38	<0.05	0.08
Misshapen eggs	0.06	0.00	0.160	0.03
Slab-sided eggs	0.06	0.00	0.160	0.03
Double yolk	0.09	0.06	0.671	0.07

[1]On: from 17 to 26 wk of age, hens were kept in pens with the nests opened with the inner led lights turned on at low intensity (10 lux) 1.5 h before the light of the installation turned on (4 pens). Off: from 17 to 26 wk of age, hens were kept in pens with the nests closed until turning on the installation light and without any light inside.

housing with respect to those pens which did not. Accordingly, a lower rate of eggs laid outside of the nest and collected on the belt or on the wire mesh of the aviary, that is, the eggs expected to break and to get dirty, was recorded in the former compared to the latter pens ($P < 0.001$; Table 3).

The training of hens with nest lighting also affected the distribution of eggs laid on the different levels of the aviary, with a higher rate of eggs laid on the first tier in the case of trained hens compared to untrained ones (48.1% vs. 46.0%; $P < 0.001$) and an opposite result for the second tier (50.4% vs. 52.7%; $P < 0.001$; Table 3). The distribution of hens at the different levels of the aviary confirmed these results about egg distribution (Table 4). In fact, the rates of hens found on the wire mesh and on the feeding perches of the first tiers were higher in pens with hens trained with nest lighting compared to pens with untrained hens (16.9% vs. 15.7% and 2.01% vs. 1.74%, respectively; $P < 0.01$ and $P < 0.05$, respectively) which also corresponded to a lower rate of hens on the wire mesh of the second tier (23.3% vs. 23.9%; $P < 0.05$) (Table 4).

Thus, the use of the nests was likely promoted by the low-intensity nest lighting during the first weeks at housing and at the start of laying, which increased the proportion of eggs laid in the nests at the start of oviposition (wk 21−26 of age; Pillan et al., 2020) and, according to the present study, later during the following laying period when nest lighting was switched off in all nests to provide hens with a suitable place for laying.

In fact, some authors (Ma et al., 2016) showed that white hens (Hy-line W-36) at 23 wk of age preferred to lay in dark places (<1 lux). However, other authors (Appleby et al., 1984) previously pointed out that the hen preference for laying in nests with or without lighting could vary with the commercial genotype (white vs. brown hens) and the sexual maturity stage (young hens at the start of laying vs. mature hens 1.0−2.5 mo after the first egg). In details, only young white hens preferred dark nests, while both young and mature brown hens chose enlightened nests, and mature white hens did not show a clear preference. Moreover, wild-living avian species (cavity nesting birds) have also been found to prefer enlightened nests for laying (Podkowa and Surmacki, 2017).

Table 3. Effect of nest lighting at housing in an experimental aviary on laying position of eggs in the aviary and laying level of eggs (average LS means values of samplings from 28 to 45 wk of age).

Variables	Nest lighting[1]		P value	SEM
	Off	On		
Recordings[2] (n)	72	72		
Laying position (% of laid eggs)				
Floor	1.30	1.53	<0.05	0.19
Belt, inside the nest	84.8	87.2	<0.001	0.8
Belt, outside the nest	11.5	9.66	<0.001	0.4
Wire mesh	2.45	1.66	<0.001	0.35
Laying level (% of laid eggs)				
Floor	1.30	1.53	<0.05	0.19
First level	46.0	48.1	<0.001	1.0
Second level	52.7	50.4	<0.001	1.0

[1]On: from 17 to 26 wk of age, hens were kept in pens with the nests opened with the inner led lights turned on at low intensity (10 lux) 1.5 h before the light of the installation turned on (4 pens). Off: from 17 to 26 wk of age, hens were kept in pens with the nests closed until turning on the installation light and without any light inside.
[2]Number of data collected along the samplings from 28 to 45 wk of age.

Table 4. Effect of early nest lighting at housing in an experimental aviary on distribution of hens (% of observed hens) in the different level of the aviary (average LS means values of samplings from 28 to 45 wk of age).

Variables	Nest lighting[1]		P value	SEM
	Off	On		
Recordings[2] (n)	144	144		
Floor (%)	36.4	35.7	0.116	1.1
First level (%)	19.7	21.1	<0.001	0.9
Wire mesh (%)	15.7	16.9	<0.01	0.8
External perches (%)	0.85	0.85	0.984	0.11
Feed perches (%)	1.74	2.01	<0.05	0.30
Nests (%)	1.45	1.33	0.248	0.15
Second level (%)	31.0	30.6	0.251	0.5
Wire mesh (%)	23.9	23.3	<0.05	0.4
Tier external perches (%)	1.24	1.40	0.072	0.20
Feed perches (%)	4.16	4.07	0.429	0.23
Nests (%)	1.67	1.80	0.144	0.14
Third level (perches)	12.9	12.6	0.231	0.3

[1]On: from 17 to 26 wk of age, hens were kept in pens with the nests opened with the inner led lights turned on at low intensity (10 lux) 1.5 h before the light of the installation turned on (4 pens). Off: from 17 to 26 wk of age, hens were kept in pens with the nests closed until turning on the installation light and without any light inside.
[2]Number of data collected along the samplings from 28 to 45 wk of age.

Thus, while the preference of hens for dark or enlightened nests has been found to change with hen ontogenetic factors, the management of the light (as in the present trial) can change the distribution of the animals in the aviary and can eventually serve to train hens to the use of nests as measured both in terms of eggs laid in the nests and between the different levels. In fact, Lentfer et al. (2013) found that white hens (Lohmann Selected Leghorn hens) in an aviary system were likely to increase the use of the nests (measured as number of eggs laid inside the nests) when the area around the nests was enlightened, despite all nests were without light inside. Additionally, Li et al. (2018) recorded that White Leghorn hens overcrowded and laid a higher number of eggs in the corner nests of the top level of an aviary, where supplementary lightening had been set to attract and move them from the lower levels.

Effect of the Color of Nest Curtain

After the peak of laying was reached, we changed the color of the nest curtains and measured, between 28 and 37 wk, any preference of hens for the yellow or red color based on differences in the distribution of laid eggs and in the distribution of hens among the different levels of the aviary.

First, results showed that the rate of laid eggs was numerically lower on the first tier compared to the second tier (on average 46.5% vs. 51.7% of total eggs;

Table 5. Effect of color of nest curtains on laying position of eggs and laying level of eggs in an experimental aviary (average LS means values of samplings from 28 to 37 wk of age).

Variables	Color of nest curtains[1]				*P* value	SEM
	RR	RY	YY	YR		
Recordings[2] (*n*)	36	36	36	36		
Laying position (% of laid eggs)						
Floor	1.63[a]	1.65[a]	1.74[a]	2.23[b]	<0.05	0.17
Belt, inside the nest	84.1	84.1	83.0	84.4	0.078	1.6
Belt, outside the nest	11.6[ab]	11.0[b]	12.1[a]	10.8[b]	<0.05	1.1
Wire mesh	2.71[AB]	3.26[A]	3.23[A]	2.54[B]	<0.001	0.52
Laying level (% of laid eggs)						
Floor	1.63[a]	1.65[a]	1.74[a]	2.23[b]	<0.05	0.17
First level	47.5[A]	43.8[B]	48.2[A]	46.6[A]	<0.001	1.1
Second level	50.9[B]	54.6[A]	50.1[B]	51.2[B]	<0.001	1.1

[a,b]LS means with different superscript letters are different ($P < 0.05$).
[A,B]LS means with different superscript letters are different ($P < 0.01$).
[1]RR: red curtains in nests of both tiers (2 pens). RY: red curtains in nests of the first tier and yellow curtain in nests of the second tier (2 pens). YY: yellow curtains in nests of both tiers (2 pens). YR: yellow curtains in nests of the first tier and the red curtains in nests of the second tier (2 pens).
[2]Number of data collected along the samplings from 28 to 37 wk of age.

Table 6. Effect of color of nest curtains on distribution of hens (% of observed hens) in the different level of an experimental aviary (average LS means values of samplings from 28 to 37 wk of age).

Variables	Color of nest curtains (C)[1]				*P* value	SEM
	RR	RY	YY	YR		
Recordings[2] (*n*)	20	20	20	20		
Floor (%)	34.1	33.3	35.3	35.5	0.057	1.3
First level (%)	22.4[A]	22.4[A]	20.2[B]	20.9[AB]	<0.01	1.1
Wire mesh (%)	18.6[a]	17.8[ab]	16.4[b]	17.2[ab]	<0.05	1.0
External perches (%)	1.02	0.87	0.85	0.95	0.582	0.17
Feed perches (%)	1.38[C]	2.60[A]	2.01[B]	1.66[BC]	<0.001	0.22
Nests (%)	1.42[A]	1.16[AB]	0.94[B]	1.06[B]	<0.01	0.12
Second level (%)	32.6	33.0	33.3	32.7	0.682	0.5
Wire mesh (%)	23.9	23.8	24.3	24.6	0.347	0.4
Tier external perches (%)	1.84[A]	1.87[AB]	1.34[B]	1.22[B]	<0.001	0.21
Feed perches (%)	3.87[b]	4.41[a]	4.54a	4.03[ab]	<0.05	0.22
Nests (%)	1.49	1.45	1.59	1.41	0.667	0.15
Third level (perches)	12.4	12.8	12.8	12.4	0.359	0.4

[a,b]LS means with different superscript letters are different ($P < 0.05$).
[A,B]LS means with different superscript letters are different ($P < 0.01$).
[1]RR: red curtains in nests of both tiers (2 pens). RY: red curtains in nests of the first tier and yellow curtain in nests of the second tier (2 pens). YY: yellow curtains in nests of both tiers (2 pens). YR: yellow curtains in nests of the first tier and the red curtains in nests of the second tier (2 pens).
[2]Number of data collected along the samplings from 28 to 37 wk of age.

Table 5), suggesting a slightly higher preference of hens for the upper levels of the aviary that is confirmed by the higher rate of hens observed on the second tier and the third-level perches compared to hens observed on the first tier (on average 32.9 and 12.6% vs. 21.5%; Table 6). Most hens on the first and second tiers were observed standing or moving on the wire mesh (on average 17.5% on the first tiers and 24.2% on the second tiers, respectively). More animals were observed on feeding perches (1.91 and 4.21% on the first and second tiers, respectively) than on the external perches of the aviary (0.92 and 1.56% on the first and second tiers, respectively). These observations could be attributed both to the different activities associated with the different types of perches during the light hours (feeding perches used for accessing feed and external perches used to move from one level of the aviary to another) and to the different availability (in terms of length) of feeding perches and external perches (4.3 cm/hen and 2.2 cm/hen, respectively) at the first and second tiers. Finally, perches of the third level were extensively used during the day (on average 12.6% of observed hens; 5.4 cm/hen available), while a few hens were in the nests (on average 1.15 and 1.86% of the first and the second tier, means of observations at 8:30 and 13:30; Table 6).

Previous studies (Odén et al., 2002; Brendler et al., 2014; Brendler and Schrader, 2016; Campbell et al., 2016b) have also observed more laying hens on the perches of the upper than the lower levels (Newberry et al., 2001), even if perch use changed according to the genotype and during the 24 h. Ali et al. (2016) observed that the rate of hens on the upper levels of the aviary was higher in white than in brown hens from 17:30 and during the night, while it was lower in the former breed as compared to the latter during the morning. Additionally, during the day, dual-purpose hens (Lohmann dual) were found to use more the lower perches, while conventional layer hens used the higher perches (Giersberg et al., 2019). Ali et al. (2019) found more white hens on the aviary ledges and perches and more brown hens on wire mesh of the aviary during the daily hours. Finally, Campbell et al. (2016c) observed more white hens on perches during nightly than during daily observations (45.1% vs. 25.5% of hens, respectively).

As for the distribution of the laid eggs between the 2 tiers, a higher rate of eggs was always found on the second tier compared to the first one with the least homogeneous distribution in RY pens compared to RR, YY, and YR pens (+10.8 vs. +3.4, +1.9, and +4.6 percentage points of eggs laid on the second tier compared to the first one, respectively; Table 5). Then, the rate of eggs laid on the floor was lower in pens with red curtains (i.e., RR and RY) on the first tier compared to the YR pens (1.63 and 1.65% vs. 2.23%; $P < 0.05$); the rate of eggs laid on the first tier was the lowest and the rate of eggs on the second tier was the highest in the RY pens compared to the other pens ($P < 0.001$) (Table 5).

Taken together, under our conditions, the results suggest a hen preference for laying at the tiers with yellow nest curtains, since the distribution of eggs between the first and the second tier was more balanced in the case of yellow curtains on all nests (YY pens). Otherwise, the rate of eggs laid on the second tier was highest when nests on this tier had yellow curtains (RY pens), and the rate of eggs on the lower levels of the aviary (i.e., floor and first tier nests) was higher when nests of the first tier had yellow curtains (48.8 and 49.9% in YR and YY pens vs. 45.5% in RY pens). These results were confirmed by the observations about the distribution of hens in the aviary during the morning, when the presence of yellow nest curtains increased the rate of hens on the floor of YY and YR pens compared to RR and RY pens (35.3 and 35.5% vs. 34.1 and 33.3%, respectively; $P = 0.05$; Table 6).

When considering the position of the laid eggs with respect to nests, a lower rate of eggs tended to be laid inside the nests in the YY pens compared to the other pens (on average −1.2 percentage points; $P = 0.08$), which corresponded to the highest rate of eggs laid outside of the nests and collected on the belt (12.1% of laid eggs; Table 5). Differences in the rate of eggs laid on the wire mesh and collected by hand by the operator according to the color of nest curtains were small despite being statistically significant. Thus, based on the results of distribution of hens, distribution of laid eggs among the different levels of the aviary, and eggs laid in the nests, hens appeared to be attracted and moved toward the levels with yellow nest curtains, although this did not result in an increase of the use of the nests for laying.

Generally speaking, results from the scientific literature regarding the preference of the domestic fowl and laying hens for the different colors shows no consistency, as reviewed by Jones et al. (2000). On one side, there is evidence that the blue color produces an adverse reaction in chickens (whatever the object given to the animals, food or strings); the red color is usually interpreted by animals as a signal of danger, whereas the yellow color (strings, nest curtains, food) attracts chickens and hens. The preference of hens for yellow nests was confirmed when hens had the possibility of choosing between nests with yellow walls, nests with plastic flaps and standard nests (i.e., wooden color walls, no curtains and wood-shavings as nesting material) (Clausen and Riber, 2012). A key role in determining the color preferences stands in the cognitive process associated with the exposure. In fact, the preference of chickens for yellow may be attributed to the color of 1-day chicks and the early exposure to this color. Nevertheless, neither the early exposure to colors other than yellow nor the preference shown by chickens in these early times for one color or another can modify the later preference of hens for the yellow color (measured as the preference for a yellow nest; Huber-Eicher, 2004). Regarding this, early exposure to red at a high light intensity exacerbated the later preference for yellow nests compared to red, blue or green nests, while early exposure to red at a low light intensity or to yellow at either a high or a low light intensity did not modify later hen preference toward nest color (Zupan et al., 2007).

CONCLUSIONS

Under our experimental conditions, the use of nest lighting in an aviary system from 17 to 26 wk of age, besides increasing egg production, was useful to train brown hens to use nests in later production stages and to balance the hen and therefore, the egg distribution between the different tiers. After the peak of egg production, yellow nest curtains attracted hens, which indicates the preference of hens toward this color compared to red, however, no positive effect on the use of nests for laying eggs was recorded at this stage. Thus, the possibility of using colored objects or colored structures to train hens to use the aviary levels should further tested when hens are first housed in the aviary. In fact, early interventions seem to be a better strategy than the correction of unbalanced distributions during later production stages. Present results need to be confirmed with additional genotypes (brown and white), as a differential expression of behaviors and preferences toward light management and colors might exist according to genetics.

ACKNOWLEDGMENTS

The PhD grant of Dr. Giulio Pillan was funded by Unismart (call 2019; grant: DOT1319383). The research project was funded by the University of Padova within the Call Unimpresa (year 2019; CUP: C22F20000020005) and the University of Padova (BIRD year 2020; CUP: C24I20000260005).

The authors wish to thank Dr. Luca Carraro (University of Padova, Legnaro, Padova, Italy) for his assistance on animal care and egg controls.

Availability of Data and Materials: The datasets analyzed in the current study are available from the corresponding author upon reasonable request.

Authors' contributions: G.X. and A.T. conceived and designed the experiment. G.P., F.B., A.P., F.P., and A. T. collected experimental data. G.P., I.E., and X.A. performed the statistical analyses. G.P., C.C., and A.T. analyzed and interpreted the data. G.P., C.C., and A.T. wrote the first draft of the manuscript. A.T. and G.X. provided funding for this project. All authors critically reviewed the manuscript for intellectual content and gave final approval of the version to be published.

DISCLOSURES

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in the present study.

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