

## Early training of hens: effects on the animal distribution in an aviary system

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The study aimed at evaluating if the training of hens at their arrival in the production farm affected the distribution of animals in the aviary. Training consisted in raising by hand animals found on litter after turning off the light during the first two weeks. A total of 1,800 hens, aged 17 weeks, were allocated in 8 pens of the aviary and assigned to the trained or untrained groups. From 18 to 26 weeks of age, two operators recorded the number of animals on the different parts of the aviary at two observation hours (morning and afternoon). The training decreased the rate of hens on the floor (23.5% vs. 24.5%;  $P < 0.05$ ) and increased the rate of those on the third level (9.26% vs. 8.73%). The rate of animals on the floor (24.4% vs. 23.6%;  $P = 0.05$ ) and on the second tiers (36.9% vs. 33.2%;  $P < 0.001$ ) was significantly higher at morning hours compared to afternoon, whereas the rate of animals on the first tiers (29.6% to 33.7%;  $P < 0.001$ ) and on the perches of the third level (8.84% to 9.25%;  $P < 0.05$ ) was lower. As the age advanced, the rate of hens on the floor significantly increased (21% to 25% from week 18 to 26); animals at the first tiers decreased from week 18 (35.3%) to weeks 20-25 to reach the minimum value at week 26 (27.9%); differences in animals on the second tiers were erratic; rate of animals on the third level was the lowest (7.13%) at week 18 and the highest (11.7%) at week 26.

**Keywords:** aviary, laying hens, space use, nest lighting, observation hour

### 1 Introduction

Cage-free systems have been largely implemented during the last years to comply animal welfare requirements, with special emphasis on the possibility of expressing a complete behavioural repertoire, such as freedom of movement, nesting and dust bathing behaviours (Vestergaard, 1992, Colson et al., 2007). Nevertheless, these systems are not yet fully developed and their use has brought some problems for animal health and welfare as well as egg production (Janczak and Riber, 2015). In fact, the aviary design varies according to equipment manufacturers; housing details and management can differ among systems, which could affect how animals distribute themselves in the available space as well as their nesting behaviour (Channing et al., 2001). These issues can in turn affect animal distribution in the aviary, causing injuries or death due to overcrowding and suffocation (Ali et al., 2016), as well as laying eggs out of the nest, which increases discarded eggs (Mathews and Sumner, 2014). The capability of hens coming from a cage system to adapt into a cage-free system at transfer time is the first crucial step (Yang et al., 2018, Janczak and Riber, 2015). It depends on

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several factors, such as genotype (Ali et al., 2016), age at the transfer time (MacLachlan et al., 2020), or access time to the floor litter (Oliveira et al., 2019). According to genetic producers, training layer hens to sleep at their arrival on the different levels of the system can improve space use and reduce subsequent egg laying on the ground (Hy-Line, 2016). Moreover, based on existing knowledge about hen physiological and behavioural response to different lighting conditions (Kjaer and Vestergaard, 1999, Ma et al., 2016, Li et al., 2018), the presence and intensity of the light could also modulate the distribution of animals within an housing system, whereas the nest lighting could affect the nest entry and exit of the hens.

Thus, the present research aimed at evaluating if the training of hens at their arrival in the farm, i.e. raising by hand animals found on the floor after turning off the light during the first two weeks, affected the number of animals on the floor at night during the same period as well as the distribution of animals in the aviary during the day in the following period until 26 weeks of age. The effect of nest lighting on animal distribution was also studied.

## 2 Material and methods

### 2.1 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 regarding the protection of animals used for experimental and other scientific purposes. Research staff involved in animal handling were animal specialists (PhD or MS in Animal Science) and veterinary practitioners.

### 2.2 Housing, animals, and system management

The trial was performed at the Experimental Farm "Lucio Toniolo" of the University of Padova (Legnaro, Padova, Italy). The building was equipped with cooling system, forced ventilation, radiant heating and controlled light systems. The aviary consisted of two tiers, equipped with collective nests (1 nest/60 hens) with plastic curtains, perches, nipple drinkers and automatic feeding, and a third level with only perches and feeders. The aviary had a total dimensions of 2.50 m width × 19.52 m length × 2.24 m height. Free space below the aviary was 5.70 m width × 19.52 length. The aviary was divided in 8 pens of 2.44 m length each.

A total of 1,800 hens (1,448±95.2 g) Lohmann Brown-Classic (Lohmann Tierzucht GmbH), aged 17 weeks, were delivered by an authorized truck from a commercial farm to the experimental facilities. On arrival, hens were randomly allocated in the 8 pens of the aviary (225 hens per pen; 9 hens/m<sup>2</sup> available surface) and assigned to two experimental groups (4 pens per group): 1) the control group (C), hens not submitted to any training; and 2) the trained group (T), hens raised by hand from the ground to the different levels of the system after turning off the light during the first 15 days. Within the two groups (C and T), half pens had the nest opened with the inner led light turned on at low intensity (10 lux) 1.5 h before the light of the installation turned on; the other half had the nests closed until turning on the installation light and without any light inside.

During the trial, minimum and maximum temperature inside the farm averaged 18.8±1.1°C and 20.4±0.5°C, respectively; minimum and maximum relative humidity averaged 30.3±10.6% and 52.9±6.1%, respectively. Ten hours of light in the stable were provided during the first week following the arrival of pullets, which increased at 14 h of light after 4 weeks to remain stable.

From arrival until 18 weeks of age, hens were fed *ad libitum* with a commercial diet containing 18.1% crude protein, 3.8% crude fat, 3.9% crude fibre, 8.9% ash, 1.9% calcium, 0.71% phosphorus. From 19 until 20 weeks of age, hens received a diet containing 18.0% crude protein, 4.1% crude fat, 3.8% crude fibre, 10.3% ash, 2.5% calcium, 0.72% phosphorus. From 21 until 26 weeks of age, hens received a diet containing 18.1% crude protein, 5.4% crude fat, 3.3% crude fibre, 13.7% ash, 4.2% calcium, 0.63% phosphorus.

### 2.3 In vivo recordings

For the purpose of the present study, hens were monitored from their arrival until 26 weeks of age. During the first two weeks (training period), 30 min after turning off the lights of the installation, an operator entered the pens wearing red head-light and recorded the number of pullets on the ground

and on the lowest external perch of the first tiers (50 cm high) in all pens. Thus, the hens of the trained group were gently put inside the aviary whereas those of the untrained group were left as they were.

Additionally, from 18 to 26 weeks of age, two operators scored the number of animals on the different levels of the aviary (floor, first and second tiers, perches of the third level) at two observation hours (8.30 and 13.30) by direct observation.

Finally, during the trial and from the 21<sup>st</sup> week of age, the rate of egg deposition (% of present hens) was recorded three times a week per each pen.

## 2.4 Statistical analysis

The number of hens found on the floor and the lowest external perches 30 min after turning off the lights of the installation and during the first 15 days of housing was analysed using a mixed model that included, as main effects, the pullet management (trained vs. untrained), the nest lighting system (on vs. off), the day of observation and all their interactions, whereas the pen was a repeated measurement.

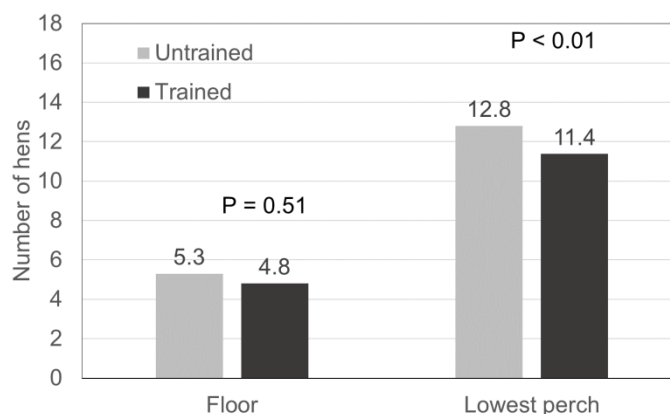
The percentage of hens observed from week 18 to week 26 in the different levels of the aviary was analysed through a mixed model that included, as main effects, the management (trained vs. untrained hens), the nest lighting system (on vs. off), the age of hens, the observation hours (morning vs. afternoon) and all their interactions, whereas the pen was a random effect.

The weekly egg deposition (% present hens from week 21 to week 26) was analysed using a mixed model that included, as main effects, the pullet management, the nest lighting system, the age of hens, and their interactions, whereas the pen was a repeated measurement. The PROC MIXED of SAS (Statistical Analysis System, 2013) was used for all analyses.

Differences among means with  $P < 0.05$  were assumed to be statistically significant. The Bonferroni's t-test was used to compare the least squares means.

## 3 Results and discussion

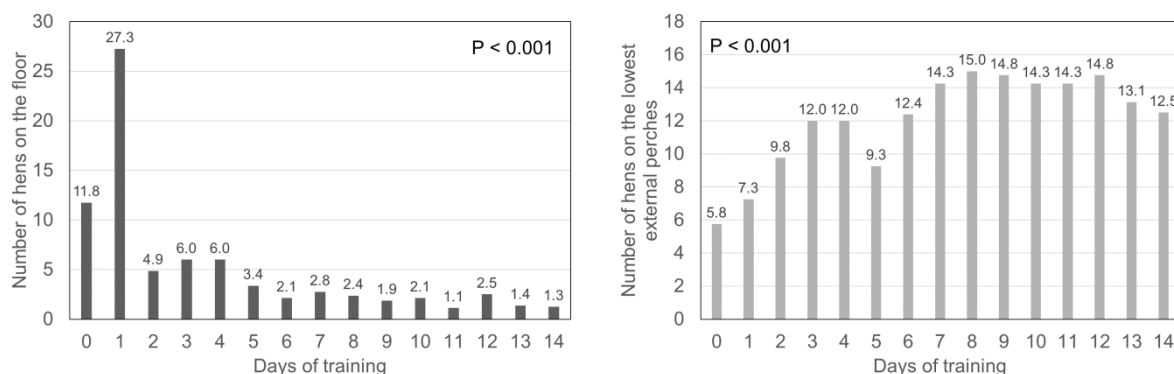
The average number of hens found on the floor after turning off the light and during the first 15 days after housing was not affected by the training treatment, whereas the number of hens on the lowest external perches was significantly lower in trained compared to untrained hens (Figure 1). During observations (Figure 2), the number of hens found on the floor significantly increased from the first to the second day after housing (11.8 to 27.3) which could be related to a first exploration activity, but soon decreased on the third day to 4.9 to reach low and stable values after one week.



**Figure 1** Number of hens (average of the first 15 days after housing) found on the floor and on the lowest external perches of the pens in the aviary after turning off the light. Trained group: hens on the floor and the lowest external perches had been raised on the aviary during the first 15 days. Untrained hens were left as they were

No significant interaction was recorded between training and observation day (data not shown), which means that the number of hens on the floor decreased regardless from the training treatment. On the other hand, the number of hens on the lowest external perches soon increased after the first two days

of observation, likely because hens raising from the floor at dusk stopped on the first level. Then, the reduction of hens on the lowest external perches at the last two observations could be explained by a higher ability and aptitude of the animals to reach upper levels.



**Figure 2** Number of hens (average of trained and untrained hens) found on the floor (left) and on the lowest external perches (right) of the pens in the aviary after turning off the light during the first 15 days after housing (i.e. 15 days of training).

In the present study, the average distribution of animals within the different parts of the aviary was rather balanced to the available surfaces on the floor, the two tiers and the perches of the third level and to the availability of feeders on all levels, and drinkers and collective nests on the two tiers. On average, 24% of animals were found on the floor, 32% on the first tiers, 35% on the second tiers and 9% on the perches of the third level (Table 1). Differently, Odén et al. (2002) observed most hens on the top of the two systems they compared (a multi-tier system and an a-shaped perch system). In our study, the balanced distribution of the animals in the aviary could have been partly related to the strain used. In fact, between 25 and 28 weeks of age, Ali et al. (2019a) found a higher number of brown hens on wire floors whereas a higher number of white hens was found on elevated perches and ledges.

On average of all weeks of observations (18 to 26 weeks of age), the distribution of animals within the different part of the aviary was partly affected by the early training of pullets to sleep on the tiers. In details, the rate of hens found on the floor was lower in trained vs. untrained hens (23.5% vs. 24.5%;  $P < 0.05$ ), whereas the rate of those on the perches of the third level was higher in the former compared to the latter (9.36% vs. 8.73%) (Table 1). On the other hand, the rates of hens found on the first and the second tiers did not significantly differ. Thus, the early training somewhat pushed animals to stay on the aviary rather than on the ground and permitted an early exploration until the third level on which more animals were observed in the trained compared to the untrained group. According to Ali et al. (2019b), training hens to the use of the aviary (by early exposure to perches) affected their use of the aviary at least for the first 10 weeks after housing.

The effect of the nest lighting on animal distribution in the aviary was similar to that of the early training. In fact, the rate of hens found on the floor (23.1% vs. 24.9%;  $P = 0.01$ ) and on the first tiers (31.2% vs. 32.1%;  $P = 0.01$ ) was lower in pens with the nests opened and lightened before the general light turned on. On average, during oviposition weeks, the oviposition rate was higher in the case of nest lighting (63.3% vs. 60.0%;  $P < 0.001$ ), which means that the higher number of light hours provided by nest lighting stimulated oviposition. In the present trial, the nest lighting also reduced the number of hens observed on the floor and stimulated them to reach the first tiers. Indeed, a previous study (Appleby et al., 1984) found that the choice of the nest during oviposition based on the nest lighting (on vs. off) largely varied with both strain and maturity; this means that more knowledge is necessary about the lighting conditions (e.g. presence, duration, intensity) and according to the different management and housing of each farm and each system.

Whether the reduction of animals found on the floor area during the first period of oviposition, as observed in the present trial with early training and nest lighting, could be considered a positive result, it needs to be confirmed during the whole production cycle by measuring the rate of eggs laid on the floor and out of the nests. In fact, the overall mean weekly percentage of floor eggs in an aviary with free access to litter averaged  $4.15 \pm 1.53\%$  during the whole production cycle (until 76 weeks of age) and reached 10-12% during some periods (Oliveira et al., 2019). Besides, the percentage of eggs laid out of nests can change with the housing system (17.6% in aviary systems compared to 9.6% in

furnished cages; Hunniford and Widowski, 2016) as well as housing details (e.g. space availability, enrichments) within the same system (in furnished cages: from 6.8 until 21.9%; Hunniford et al., 2014, 2017, Hunniford and Widowski, 2017). Nevertheless, in our study, differences in the rate of animals found on the floor during the first period (until 26 weeks of age) were rather low in absolute values (1-2 percentage points) which could have a limited impact on the rate of eggs laid on the litter in the following oviposition.

**Table 1** Weekly oviposition (% of present hens) and distribution of hens (% of observed animals) in the different parts of the aviary according to training, nest lighting, week of age and hour of observation

	Floor (%)	First tiers (%)	Second tiers (%)	Perches of the third level (%)	Oviposition (%)
Early training					
Untrained	24.5	31.3	35.2	8.73	62.6
Trained	23.5	31.9	34.9	9.36	60.7
Nest lighting					
On	23.1	31.2	35.2	9.24	63.3
Off	24.9	32.1	34.9	8.84	60.0
Observation hour					
Morning (8.30)	24.4	29.6	36.9	8.84	-
Afternoon (13.30)	23.6	33.7	33.2	9.25	-
Week of age					
W18	21.0 <sup>a</sup>	35.3 <sup>d</sup>	36.3 <sup>c</sup>	7.13 <sup>a</sup>	-
W19	22.3 <sup>ab</sup>	33.2 <sup>c</sup>	35.7 <sup>bc</sup>	8.43 <sup>a</sup>	-
W20	23.0 <sup>ab</sup>	31.9 <sup>bc</sup>	35.6 <sup>bc</sup>	9.24 <sup>ab</sup>	-
W21	23.6 <sup>bc</sup>	31.2 <sup>b</sup>	34.6 <sup>ab</sup>	10.2 <sup>b</sup>	6.7 <sup>a</sup>
W22	25.7 <sup>c</sup>	31.6 <sup>bc</sup>	33.3 <sup>a</sup>	9.13 <sup>ab</sup>	29.5 <sup>b</sup>
W23	23.0 <sup>abc</sup>	31.4 <sup>bc</sup>	36.8 <sup>c</sup>	8.47 <sup>a</sup>	65.8 <sup>c</sup>
W24	25.8 <sup>c</sup>	31.3 <sup>bc</sup>	34.7 <sup>abc</sup>	7.99 <sup>a</sup>	85.1 <sup>d</sup>
W25	26.4 <sup>c</sup>	30.6 <sup>b</sup>	33.6 <sup>a</sup>	9.05 <sup>ab</sup>	91.1 <sup>e</sup>
W26	25.0 <sup>bc</sup>	27.9 <sup>a</sup>	35.1 <sup>abc</sup>	11.7 <sup>c</sup>	91.7 <sup>e</sup>
<i>P-values</i>					
Early training	0.02	0.12	0.37	0.001	0.12
Nest lighting	0.01	0.01	0.55	0.21	<0.001
Observation hour	0.05	<0.001	<0.001	0.02	-
Week of age	<0.001	<0.001	<0.001	<0.001	<0.001
RSD	2.69	2.35	1.97	1.28	0.96

Different superscript letters on least squares means within the same column stand for significant differences. RSD: residual standard deviation

In the present trial, animal distribution within the aviary was also largely affected both by the observation hour and the age (week) of the hens. Regarding the observation hour, the rate of animals on the floor (24.4% vs. 23.6%;  $P=0.05$ ) and the rate of animals on the second tiers (36.9% vs. 33.2%;  $P<0.001$ ) was significantly higher at the morning observation compared to the afternoon one. On the other hand, the rate of animals on the first tiers (29.6% to 33.7%;  $P<0.001$ ) and on the perches of the third level (8.84% to 9.25%;  $P<0.05$ ) was higher in the afternoon (Table 1). Indeed, hens are known to use perches and tiers to rest undisturbed (Ali et al., 2019a, 2019b, Brendler and Schrader, 2016) and

clearly prefer the highest areas for night roosting as reported by Odén et al. (2002) who also observed most of the hens on the top of the two systems they investigated. Under the conditions of our study, the higher rate of hens on the second tiers at the morning observation likely depended on the fact that hens from the third level visited and/or started to use the nests of the second tiers. Indeed, when comparing different strains between 25 and 28 weeks of age, Ali et al. (2019a) found a higher number of brown hens in nests during the morning compared to white hens, whereas these latter used the nest more extensively during the day and were in higher number in the nest at midday. In fact, in Hy-line hens at 21 weeks of age, Hunniford et al. (2017) found that the number of hens sitting and eggs laid in the nest reached a peak between 8.30 h and 9.30 h. Surely, laying pattern can change with hen genotype (Tůmová et al., 2017), but any overcrowding could increase eggs laid outside the nest because hens could not reach the nest during peak demand hours, which prevents them to perform their normal pre-laying behaviour and cause frustration, thus affecting their welfare (Kruschwitz et al., 2008).

Regarding the week of age, significant differences were found for all observations ( $P < 0.001$ ). In details, the rate of hens found on the floor significantly increased with age (from 21% at week 18 to 25% at week 26); animals standing at the first tiers decreased from week 18 (35.3%) to weeks 20-25, to reach the minimum value at week 26 (27.9%); differences in animals in the second tiers, despite significant, showed erratic changes among the different weeks; finally, the rate of animals on the third level of the aviary showed the minimum values on the first week of observation (7.13% on week 18) and the maximum one on the last week of observation (11.7% on week 26).

Indeed, main changes in animal distribution according to age were observed between weeks 18-20 and the following weeks which is likely to be associated both to the increased familiarity of the hens with the system and with the start of oviposition. In fact, during the week 21 of age deposition rate started at 6.7%, and quickly increased until 91-92% at weeks 25 and 26 of age. On the other hand, according to some authors, when age increases, hens may be less motivated to explore the aviary and its upper levels because their weight increases (Stratmann et al., 2015, Sibanda et al., 2020). Indeed, along the whole production cycle, Channing et al. (2001) found that older birds spent more time on the floor areas and less time on perches.

#### 4 Conclusions

Early training of hens to rest on the aviary affected the distribution of animals during the first period (until 26 weeks of age), as decreased rate of animals on the floor and increased rate of animals on the perches of the third level were observed. Thus, training on arrival stimulated hens to explore the aviary and to reach its upper levels early, but differences were rather low in absolute values. Indeed, when hens are moved to the farm at 17-18 weeks of age, as in the present trial, hen distribution in the different part of the aviary was rather balanced to the available surface and resources. In conclusions, whether the training of hens is effectively useful and convenient in view of the associated labour has to be confirmed in terms of laying pattern during the following oviposition cycle, with special emphasis on egg laid on the floor and out of the nests.

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