Applied Animal Behaviour Science 274 (2024) 106247

Contents lists available at ScienceDirect

Applied Animal Behaviour Science

journal homepage: www.elsevier.com/locate/applanim

# Effects of housing systems on behaviour and welfare of autochthonous laying hens and a commercial hybrid

Edoardo Fiorilla [a], Laura Ozella [a,*], Federico Sirri [b], Marco Zampiga [b], Raffaela Piscitelli [b], Martina Tarantola [a], Patrizia Ponzio [a], Cecilia Mugnai [a]

[a] Department of Veterinary Sciences, University of Turin, Grugliasco, TO, Italy
[b] Department of Agricultural and Food Sciences University of Bologna, Ozzano dell'Emilia, BO, Italy

A R T I C L E I N F O

*Keywords:*
Laying hens
Local chicken breeds
Free-range
Animal behaviour
Animal welfare

A B S T R A C T

The welfare of laying hens is crucial for a sustainable and ethical food industry. Free-range systems offer a more natural and stimulating environment, while dual-purpose autochthonous breeds and crossbreeding could address ethical concerns and improve productivity. In this study, behaviour, plumage status, and leg health of five genotypes (two local breeds, two crossbreeds with a medium growth hybrid, and one commercial hybrid) in two different housing systems (enriched cage/free-range) were evaluated. The animals were filmed with professional cameras for three consecutive days at 30 and 64 weeks of age. Each day the recording session lasted 20 minutes, resulting in a total of 120 minutes per pen (20 min X 6 days recorded). A total of 16 behaviours divided into six categories (feeding, movement, social, resting, comfort, stretching) were scored. In addition, the tonic immobility test was performed, and the plumage status and footpad dermatitis of the birds were assessed. The results revealed a significant influence of the housing system on the behaviour, plumage status, and foot health of laying hens. While the housing system affected all behaviours, mainly feeding and comfort behaviours were influenced by the genotype of the hens and the interaction between the housing system and genotype. All genotypes displayed a similar behavioural pattern within the two housing systems, except for the commercial hybrid, which differed in its behaviour from the others adapting better to the enriched cage environment. In particular, the free-range system provided hens with more opportunities to engage in movement and comfort behaviour, and social interactions. Furthermore, hens in the free-range system exhibited better plumage conditions and lower rates of footpad dermatitis, indicating a positive impact on their overall welfare.

## 1. Introduction

Studying chicken behaviour is important for several reasons. First and foremost, chickens are an important source of food for humans, and understanding their behaviour can enhance their welfare and productivity. Studying how these animals interact with each other and with their environment enables the development of more effective animal management strategies, promoting their health and mitigating stress. The welfare of laying hens is a critical factor for a modern, sustainable, and ethical food industry. Proper management practices to promote welfare can prevent the spread of diseases, reduce stress, and improve egg quality traits (Janczak and Riber, 2015). By providing adequate space, feed, water, and environmental enrichment, laying hens can perform natural behaviours and maintain their physical and welfare status. This can contribute to a reduction in the mortality rate and even to an increase in productivity (Rodenburg et al., 2008). Furthermore, guaranteeing the welfare of laying hens is also important from an ethical and human point of view as well as to satisfy the requests from consumers that are increasingly interested in buying products with both high ethical standards and nutritional value (Vale et al., 2019). The European Union's Directive 1999/74/EC, enforced in 2012, banning conventional cages for egg-laying hens, underscores the imperative for alternative non-cage systems in poultry farming to comply with welfare standards.

Free-range systems for laying hens offer an alternative to intensive cage-systems, providing hens with a more natural and stimulating environment. These systems typically include open grass, shade, and shelter, allowing hens to roam freely, engage in natural behaviours like foraging and dust bathing, develop social interactions, and reduce stress (Campbell et al., 2021). The primary challenges of free-range systems

* Corresponding author.
*E-mail address:* laura.ozella@unito.it (L. Ozella).

https://doi.org/10.1016/j.applanim.2024.106247
Received 23 December 2023; Received in revised form 3 April 2024; Accepted 4 April 2024
Available online 6 April 2024

are the greater space requirements and the need for more complex management compared to intensive systems (Campbell et al., 2020).

In egg production industry, the culling of male chicks poses a significant ethical and sustainability issue. The potential use of local breeds as dual-purpose lines could help solve this problem. Indeed, while females from dual purpose breeds can be used for egg production, males can be raised for producing meat, thus providing an alternative to the killing of day-old male chicks (Bruijnis et al., 2015). The local breeds have been developed and adapted over generations to the local climate and production conditions, making them suitable for small-scale farming systems (Stefanetti et al., 2023). Furthermore, the use of indigenous breeds can also help diversify local food systems, reducing the dependence on imported breeds and genetic resources and improving food security (Fiorilla et al., 2023). Crossbreeding chickens involves mating different breeds to create offspring with desirable traits, such as improved egg production, growth rate, and disease resistance. This enhances adaptability to various conditions, making them cost-effective for producers. While hybridization can diversify the gene pool and boost resilience, careful consideration of potential drawbacks, like reduced heterozygosity and loss of genetic resources, is crucial. Responsible and sustainable breeding programs are essential for reaping the benefits without adverse effects (Besbes and Gibson, 1999).

Understanding complex behavioural datasets often requires advanced statistical techniques. The t-Stochastic Neighbor Embedding (t-SNE) method efficiently reduces data dimensionality while preserving its structure, facilitating visualization of intricate patterns. By mapping high-dimensional data to lower dimensions, t-SNE unveils relationships and clusters, especially beneficial for nonlinear data. When coupled with two-way analysis of variance (ANOVA) to detect group differences, t-SNE enhances analysis by visually representing data, aiding in identifying distinct behavioural patterns or clusters, thus enabling a comprehensive understanding of the dataset's structure and relationships.

In this study, the behaviours, plumage conditions, and leg health of five different genotypes of laying hens were assessed. These genotypes included a commercial hybrid, two local Italian dual-purpose breeds, and their crossbreeds with a medium-growing genotype (Sasso, (Sasso Management Guide, 2022)). Local chicken breeds have proven to be highly adaptable to low-input and alternative farming systems. Their resilience and efficiency minimize the need for artificial inputs, offering a concrete alternative to reduce the environmental footprint of poultry farming while preserving biodiversity (Fiorilla et al., 2024; Mancinelli et al., 2023). Our primary aim was to evaluate how housing systems, genotype, and animal age influence the health and behaviours of laying hens. Laying hens display diverse behaviours depending on the type of housing system they inhabit. Hens kept in cage systems often exhibit a higher frequency of discomfort indicators (Lay et al., 2011). We hypothesize distinct differences between the two housing systems among genotypes, particularly between the local breeds and the commercial hybrid. Moreover, an interaction effect between genotype and housing system is expected.

## 2. Materials and methods

### 2.1. Animals and housing

The present study was conducted at the poultry facility of the Department of Agricultural and Food Sciences of the University of Bologna (Italy) and was approved by the Ethical Committe (Prot. ID: 1102/2019). Three hundred chicks of five different genotypes, Robusta Maculata (RM, 60 hens), Bionda Piemontese (BP, 60 hens), RM x Sasso (RMxS, 60 hens), BP x Sasso (BPxS, 60 hens) and a commercial hybrid (Lohmann Brown, LB, 60 hens) were used for the present study. The chicks were immunized against Marek and Newcastle diseases and sexing was determined through visual examination of the cloaca at hatching. As dual-purpose breeds, the males from the two local breeds and their crossbreed were involved in a fattening trial conducted at the University of Turin (Prot. ID: 251833), while the females were utilized in this trial, ensuring that no chicks were culled at hatching. At 18 weeks of age, the hens were randomly allocated to two different housing systems: enriched cage and free-range system. Each one of the five genotypes was divided in three replicates, for a total of 15 pens in the enriched cage farming system and 15 in the free-range farming system. The enriched cages had a total of 75 hens, 5 in each pen with 0.144 $m^2$ available floorspace each hen while the free-range farming system had a total of 225 hens, 15 in each pen with 1.067 $m^2$ available floorspace each hen as shown in Table 1 and figure S1. The dimension of the enriched cages was 120 ×60 cm, each cage had a 30 ×30 cm nest and three 60 cm perches. A feeder measuring 120 cm was positioned in front of each cage (supplementary material, Figure S2). Lighting was regulated to provide 16 hours of light and 8 hours of darkness daily, while maintaining an average temperature of 23°C.

In the free-range system, the birds were housed in fenced pens with an insulated nest for egg-laying. The pens measured 4 ×4 m, and the nests measured 140 ×120 cm (supplementary material, Figure S2). The entire facility, including the outdoor areas, was safeguarded from wild birds and predators using fences.

The trial took place from September 2022 to June 2023 in Ozzano dell'Emilia (Bologna, Italy). Throughout the entire experimental period, the birds were subjected to natural temperature, lighting, and humidity conditions. Details regarding the average temperature, rainfall in millimeters, and hours of daylight recorded in Ozzano dell'Emilia (Bologna, Italy) during the experimental trial can be found in Table S1 in the supplementary material. Water was provided *ad libitum* through a single large drinker that allowed for the simultaneous drinking of four birds.

The feed provided was a standard commercial feed designed for layers and utilized in both the enriched cage and free-range systems. It was provided in four different formulations tailored to the hen's age: Pre-Layer (18–25 weeks), Layer 1 (26–51 weeks), Layer 2 (52–62 weeks) and Layer 3 (63–66 weeks) (Table S2 supplementary material). Throughout the entire experimental trial, the birds' health status and mortality were checked daily.

### 2.2. Behavioural observations

The animals were recorded using professional cameras (Sony FDRAX43A 4 K) for three consecutive days, for a total of 60 minutes per pen at 30 weeks (20 minutes each day) of age and 60 minutes per pen at 64 weeks of age (20 minutes each day). The recordings were performed every day at the same time at 11 a.m. The camera was placed outside the pen allowing to monitor all the animals' behaviours. The recorded videos were analyzed using the Behavioural Observation Research Interactive Software (BORIS, v 7.9.7) (Friard and Gamba, 2016). The behaviours frequencies were evaluated as the number of times that a specific behaviour occurred in the pen during the daily 20 minutes periods of observations. The behaviours were divided according to a typical laying hen behaviour ethogram (Table 2). The ethogram was elaborated starting from existing literature and adapted to the specific rearing conditions of this trial (Biasato et al., 2022; Hurnik and Webster, 1990; Kenny et al., 2017; Liu et al., 2020; McCowan et al., 2006).

**Table 1**
Experimental design of trial and animal distribution in the farming systems.

| Genotype | Enriched cage (0.144 $m^2$/hen) | Free-Range (1.066 $m^2$/hen) |
|---|---|---|
| Lohman Brown | 3 pens with 15 hens/pen | 3 pens with 5 hens/pen |
| Bionda Piemontese | 3 pens with 15 hens/pen | 3 pens with 5 hens/pen |
| Robusta Maculata | 3 pens with 15 hens/pen | 3 pens with 5 hens/pen |
| Bionda Piemontese × Sasso | 3 pens with 15 hens/pen | 3 pens with 5 hens/pen |
| Robusta Maculata × Sasso | 3 pens with 15 hens/pen | 3 pens with 5 hens/pen |

**Table 2**
Laying hen ethogram for behavioural classification. The ethogram is divided into six macro-categories containing specific behaviours.

| Category | Behaviour | Description |
|---|---|---|
| Feeding | Scratching | Move the litter/dirt with claws (Biasato et al., 2022) |
| | Feeding at feeders | Eating feed from feeders (Jhetam et al., 2022) |
| | Grass feeding/ object pecking | Eating grass in outdoor space/pecking object indoor (Veldkamp and Niekerk, 2019) |
| Movement | Walking | Walking at slow speed (Rieke et al., 2021) |
| | Running | Running (Veldkamp and Niekerk, 2019) |
| | Wing flapping | Open wings wide and flapping up and down while walking or running (Liu et al., 2020) |
| Social | Menacing | Initiate fight with another chicken (Kayla et al., 2023) |
| | Fighting | Fighting between two or more chickens(Veldkamp and Niekerk, 2019) |
| | Pecking | Pecking movements directed at a pen mate ( McCowan et al., 2006) |
| | Allo-preening | Social preening (Kenny et al., 2017) |
| Resting | Crouch | Sitting down (Webster, 2000) |
| | Stand | Holding standing position (Veldkamp and Niekerk, 2019) |
| Comfort | Sand bath | Rolling or moving around in dust, dry earth, or sand (Grebey et al., 2020) |
| | Self-preening | Self-feathers grooming by means of beak (McCowan et al., 2006) |
| Stretching | Leg stretching | Putting the leg in a certain position to lengthen and elongate the muscle (Biasato et al., 2022; Carvalho et al., 2022) |
| | Wing stretching | Putting the wing in a certain position to lengthen and elongate the muscle (Biasato et al., 2022; Carvalho et al., 2022) |

### 2.3. Tonic immobility test

The tonic immobility (TI) test was performed on 3 laying hens each pen at 30 and 64 weeks of age after video recording. The hens were captured from their home pen placed on their backs in a plastic cradle and restrained for 5 seconds by a trained experimenter, who placed one hand on the bird's chest and another over its head with the head hanging down. The experimenter then removed its hand and stepped aside with their eyes averted downwards (Campbell et al., 2019). A maximum of three attempts were made to induce immobility. The animals were not allowed to remain in the immobility condition for more than 3 minutes (Ferrante et al., 2005). The duration of TI was subsequently recorded, with 3 minutes representing the maximum duration observed.

### 2.4. Feather score and feet health assessment

At 30 and 64 weeks of age, after the TI test, the feather condition score and feet health were assessed on all the animals present in each pen.

Feather score was assessed based on the protocol by Tauson et al. (2006) on six body parts, including the neck, breast, cloaca, back, wing, and tail. Feather scores ranging from 1 to 5 were used to indicate the extent of coverage, with the following criteria: score 1, minimal coverage (<25% coverage); score 2, 25%–50% coverage; score 3, 50%–75% coverage; score 4, >75% coverage; and score 5, complete coverage (Lai et al., 2010).

The footpad dermatitis (FPD) was assessed and scored using the methods of Rushen, Butterworth, and Swanson: score 0 = no lesion or slight discoloration of the skin, or healed lesion; score 1 = mild lesion, characterized by superficial discoloration of the skin and hyperkeratosis; score 2: severe lesion, involving the affected epidermis, presence of blood scabs, hemorrhages, and significant swelling of the skin (Rushen et al., 2011).

### 2.5. Data analysis

The average percentage of each behaviour and the average duration (in minutes) of TI test, expressed as mean ± standard deviation, was calculated for each genotype (BP, BPxS, RM, RMxS, and LB), housing system (free-range and enriched cage), and age (30 and 64 weeks). The data were assessed for normality using the Shapiro-Wilk test. To assess the effects of genotype, housing system, age, and their interactions on behaviours and TI test, a two-way analysis of variance (ANOVA) was employed. Multiple comparisons of the means were conducted using the Duncan test, and the least significant difference was calculated.

The presence of behavioural patterns in the enriched cage and free-range systems was analyzed using an unsupervised technique for dimensionality reduction: the t-Stochastic Neighbor Embedding (t- SNE) technique. t-SNE is an unsupervised, non-linear machine learning technique primarily used for data exploration and visualizing high-dimensional data (van der Maaten and Hinton, 2008). This method allows to visualize the resulting similar data and thus, to identify behavioural patterns in the two housing systems. In particular, the focus was set on the link between the 16 analyzed behaviours and the genotype, the age, and the two housing systems with t-SNE technique. The behavioural data set was converted into a matrix of pairwise similarities, and as an input dataset was used a set of vectors where each vector described the behavioural features of each behavioural observation.

The absolute and relative frequencies for the feather and FPD scores were calculated. To assess differences based on genotype, housing system, and age, a chi-squared test was conducted.

Statistical analyses were performed using R software, Version 3.1.2, with a significance level of $p \leq 0.05$ considered statistically significant. The t-SNE technique was performed using Python Software.

## 3. Results

### 3.1. Behavioural observations

The effects of genotype and housing system on the behaviours of laying hens are reported in Table 3. The housing system had a significant impact on the behaviour of laying hens, affecting all the behaviours considered in this study ($p < 0.001$), whereas the influence of the hens' genotype and the interaction between genotype and housing system was evident only in specific behaviours.

Specifically, laying hens in the free-range system displayed higher frequencies of behaviours like 'pecking', 'fighting', 'crouch', 'self-preening', 'leg stretching', except for 'feeding at feeders' and 'wing stretching', which were performed more frequently in the enriched cage system. Significant differences were observed in various behaviours among different genotypes, including 'scratching', 'feeding at feeders', 'grass feeding and object pecking', 'running', 'allo-preening', 'fighting', 'stand', 'sand bath', and 'self-preening'. All the behaviours related to feeding category exhibited variations across genotypes. In particular, the LB demonstrated a higher frequency of 'feeding at feeders' behaviour compared to the other genotypes. Conversely, LB showed a relatively lower occurrence of 'grass feeding and object pecking'. Specifically, our analysis revealed significant differences in the 'scratching' behaviour among genotypes ($p < 0.001$), as well as in their interaction with the housing system ($p < 0.001$). RM and BP exhibited the highest percentages of 'scratching', while LB displayed the lowest percentage of 'scratching'.

Under the category movement behaviours, a significant effect of genotype and a significant interaction between genotype and housing system was only found for 'running' ($p = 0.003$ and $p < 0.001$, respectively). When considering differences among genotypes, our analysis indicated that LB exhibited a reduced frequency of 'running' behaviour, whereas RM and RMxS demonstrated higher percentages of 'running'. Social behaviours, i.e., 'allo-preening' and 'fighting,' were significantly affected by genotype ($p = 0.03$ and $p < 0.001$,

**Table 3**
Effect of genotype (G) and housing system (HS) and their interaction on percentage of behaviours. Mean ± standard deviation of the behaviours is indicated. Means with letters (a, b, ab) denote significant differences (p ≤ 0.05).

| Behaviour | Genotype (G) | | | | | System (HS) | | p-value | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | LB | BP | BPxS | RM | RMxS | Free-range | Enriched cage | G | HS | GxHS |
| Scratching | 2.51±3.72 (b) | 5.92±6.77(a) | 4.61±5.07 (ab) | 6.23±6.88 (a) | 4.38±5.16 (ab) | 9.47±4.64 (a) | 0 (b) | <0.001 | <0.001 | <0.001 |
| Feeding at feeders | 19.08±10.08 (b) | 13.27±10.31 (a) | 13.41±11.37 (a) | 13.29±10.95 (a) | 12.66±10.25 (a) | 5.21±4.16 (a) | 23.48±7.02 (b) | 0.001 | <0.001 | 0.23 |
| Grass feeding/ object pecking | 7.25±4.58 (b) | 8.42±6.47 (ab) | 9.69±6.58 (ab) | 9.46±6.26 (ab) | 10.90±7.99 (a) | 13.92±5.38 (a) | 4.37±3.37 (b) | 0.012 | <0.001 | <0.001 |
| Walking | 13.75±11.68 | 11.07±8.98 | 11.23±8.98 | 10.05±7.57 | 10.78±7.22 | 18.65±6.77 (a) | 4.10±3.52 (b) | 0.14 | <0.001 | <0.001 |
| Running | 1.88±2.60 (b) | 3.86±4.57 (ab) | 3.83±4.64 (ab) | 4.35±5.31(a) | 4.72±5.72 (a) | 7.47±4.19 (a) | 0 (b) | 0.003 | <0.001 | <0.001 |
| Wing flap | 1.99±2.99 | 1.43±2.25 | 1.25±2.08 | 1.43±2.25 | 1.44±2.23 | 3.02±2.61 (a) | 0 (b) | 0.40 | <0.001 | 0.14 |
| Menacing | 0 | 0.10±0.49 | 0.04±0.33 | 0.19±0.71 | 0.08±0.48 | 0.16±0.65 (a) | 0 (b) | 0.14 | <0.001 | 0.13 |
| Allo-preening | 0.91±1.57 (b) | 2.03±2.92 (ab) | 1.85±2.79 (ab) | 1.98±2.74 (ab) | 2.14±2.86 (a) | 3.57±2.76 (a) | 0 (b) | 0.03 | <0.001 | <0.001 |
| Pecking | 4.41±4.97 | 3.20±3.60 | 4.48±4.39 | 3.80±3.78 | 3.48±3.89 | 1.41±1.54 (a) | 6.34±4.49 (b) | 0.26 | <0.001 | 0.051 |
| Fighting | 0 (b) | 3.36±4.26 (a) | 2.97±4.36 (a) | 3.26±4.41 (a) | 2.94±4.09 (a) | 0 (a) | 5.02±4.43 (b) | <0.001 | <0.001 | <0.001 |
| Crouch | 10.20±8.56 | 9.03±7.82 | 7.98±6.44 | 7.54±6.78 | 9.24±7.76 | 3.03±2.49 (a) | 14.57±6.38 (b) | 0.22 | <0.001 | 0.08 |
| Stand | 20.23±10.22 (b) | 15.60±7.54 (a) | 15.82±8.76 (a) | 15.58±6.76 (a) | 14.59±6.09 (a) | 20.55±8.00 (a) | 12.18±5.99 (b) | <0.001 | <0.001 | <0.001 |
| Sand bath | 1.72±2.54 (b) | 3.28±3.96 (ab) | 3.48±4.15 (ab) | 3.86±4.62 (a) | 3.52±4.30 (ab) | 6.35±3.62 (a) | 0 (b) | 0.01 | <0.001 | <0.001 |
| Self-preening | 5.36±4.49 (b) | 9.31±6.60 (a) | 9.73±6.85 (a) | 8.74±6.56 (a) | 9.44±6.24 (a) | 5.52±4.29 (a) | 11.51±6.72 (b) | <0.001 | <0.001 | <0.001 |
| Leg stretching | 3.86±5.18 | 3.57±4.81 | 3.26±4.70 | 3.29±4.63 | 2.80±3.97 (a) | 0 (b) | 6.72±4.58 | 0.72 | <0.001 | 0.37 |
| Wing stretching | 6.76±5.74 | 6.46±6.32 | 6.29±6.24 | 6.89±6.74 | 6.81±6.47 | 1.61±1.61 (a) | 11.67±5.07 (b) | 0.97 | <0.001 | 0.72 |

LB: lohman brown; BP: bionda piemontese; BPxS: bionda piemontese x sasso; RM: robusta maculata; RMxS: robusta maculata x sasso.

respectively). LB spent less time in social behaviours compared to the other genotypes. Additionally, the interaction effect between genotype and housing system significantly influenced these behaviours ($p < 0.001$). This suggests that the influence of genotype on these social behaviours may vary based on the specific housing environment, indicating a potential interplay between the environmental context and the genetic traits of the hens. In terms of resting category, the 'crouch' behaviour was primarily influenced by the housing system, while the 'stand' behaviour was affected by both genotype and housing system ($p < 0.001$). Specifically, LB displayed the highest percentage of the 'stand' behaviour compared to the other genotypes. Both the comfort behaviours (i.e., 'sand bath' and 'self-preening') were influenced by the genotype ($p = 0.01$ and $p < 0.001$, respectively) and its interaction with the housing system ($p < 0.001$), with LB that showed the lowest percentage of these behaviours. No significant differences were found between the age of the animals in terms of the percentage of behaviours. For a detailed analysis of the percentages of behaviour including the age and their interactions, refer to the Table S1 reported in the supplementary

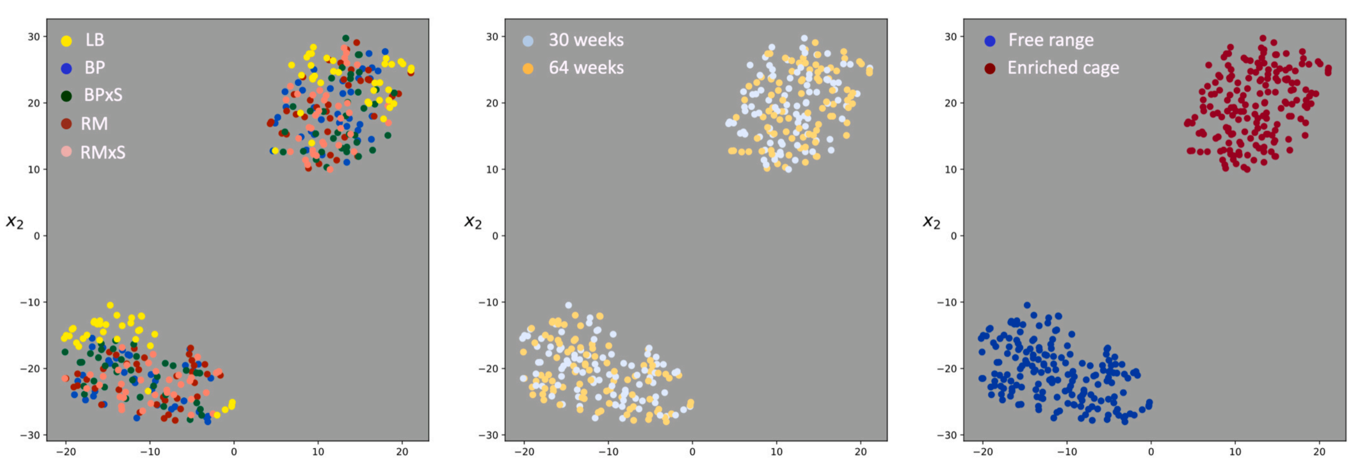


**Fig. 1.** Behavioural patterns of laying hens using t-Distributed Stochastic Neighbor Embedding (t-SNE) to map high-dimensional behavioural vectors to a 2-dimensional feature space X1 and X2 axes). Each point corresponds to a behavioural observation. Observations with similar behavioural vectors are mapped to neighboring points in the plane. Points (observations) are color coded according to genotypes (left panel), age of animals (middle panel), and housing systems (right panel). LB: lohman brown; BP: bionda piemontese; BPxS: bionda piemontese x sasso; RM: robusta maculata; RMxS: robusta maculata x sasso.

material.

### 3.2. t-SNE behavioural pattern

The influence of the housing system on behavioural patterns was also demonstrated using the t-SNE technique. This method processed each behavioural observation into a feature vector containing the frequency of the 16 observed behaviours. These 16-dimensional vectors were then visually represented using t-SNE, a dimensionality reduction technique that transforms the 16-dimensional data into a more easily interpretable 2-dimensional space (X1 and X2 axes; see Fig. 1). This visualization aimed to provide a clearer understanding of how the housing system influenced the overall behavioural characteristics of the hens. Two defined clusters of observations with similar behavioural patterns are visible. The top-right cluster contains all the observations registered for the enriched cage system, and the bottom-left cluster contains all the observations registered for the free-range system (Fig. 1, right panel). The clear separation into distinct clusters suggests a notable divergence in behavioural patterns between the enriched cage and free-range systems. On color coding by genotype, both clusters contain the five genotypes, and LB, in both clusters is the genotype that shows more similar behaviours (Fig. 1, left panel, yellow points). Each cluster exhibits closely grouped yellow dots representing LB, evident in both the free-range and enriched cage clusters. This indicates that LB shows the highest similarity in behaviours across both systems.

On color coding by age, similar behaviour patterns are not identified (Fig. 1, middle panel). In Fig. 2, the frequency and the type of every single behaviour displayed by the animals is highlighted (darker color shows higher frequency than light color). In the free-range system, the behaviours belonging to the movement category (i.e., 'walking', 'running' and 'wing flap') are performed more frequently. On the contrary, the behaviours belonging to the stretching category (i.e., 'leg stretching' and 'wing stretching') and social behaviours as 'pecking' and 'fighting', are performed more frequently in the enriched cage system.

### 3.3. Tonic immobility test

Table 4 presents the results of the statistical analysis for the TI test. The average duration of TI, measured in minutes and expressed as mean ± standard deviation, is provided for each attempt. No significant

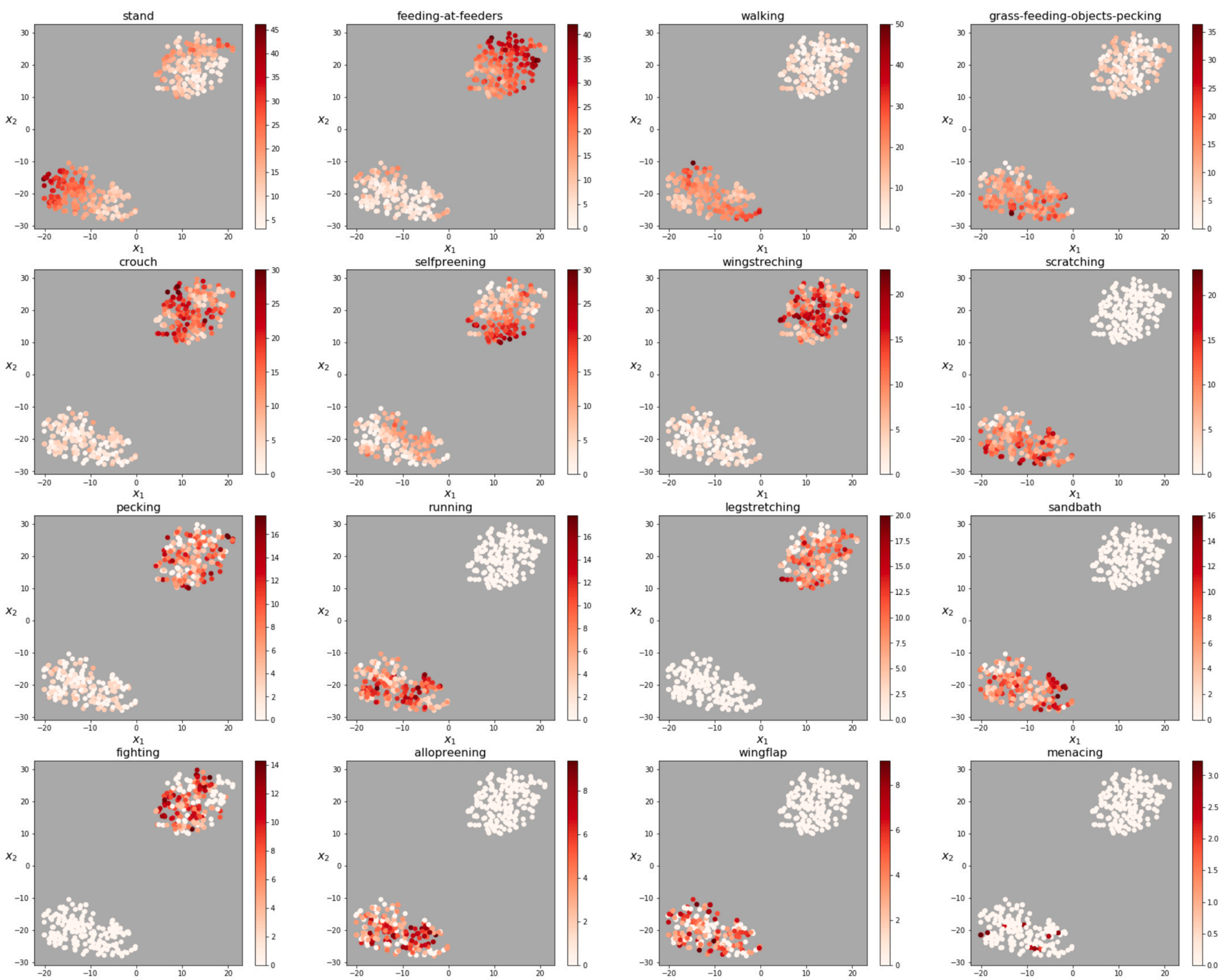


**Fig. 2.** Behavioural patterns of laying hens using t-Distributed Stochastic Neighbor Embedding (t- SNE) to map high-dimensional behavioural vectors to a 2-dimensional feature space X1 and X2 axes). (Top cluster enriched cage system, bottom cluster free-range system) Each point corresponds to a behavioural observation. Observations with similar behavioural vectors are mapped to neighboring points in the plane. The frequency and type of each behaviour exhibited by the hens are depicted, with darker colors indicating higher frequencies and lighter colors indicating lower frequencies. Behaviours are sorted from most to least performed by the animals.

**Table 4**
Effect of age and housing system (HS) and their interaction on tonic immobility test attempts. Mean and standard deviation of the minutes are indicated. Significant p-values are indicated in bold. Means with letters (a, b) denote significant differences (p ≤ 0.05).

| Tonic immobility attempt | Age | | Housing System (HS) | | p-value | | |
|---|---|---|---|---|---|---|---|
| | 30 weeks | 64 weeks | Free-range | Enriched cage | Age (A) | HS | AxHS |
| First | 0.42±0.96 | 0.63±1.17 | 0.53±1.09 | 0.51±1.05 | 0.19 | 0.92 | 0.480 |
| Second | 0.89±1.30(a) | 1.32±1.36(b) | 1.27±1.36 | 0.94±1.32 | 0.028 | 0.09 | 0.003 |
| Third | 1.09±01.32(a) | 0.48±1.06(b) | 0.65±1.18 | 0.92±1.27 | <0.001 | 0.14 | 0.010 |

differences were observed among the five genotypes in the duration of the TI test for each attempt. However, when considering the age of the laying hens, a significant effect was found on the second and third attempts. At 64 weeks, the birds displayed a longer duration of TI during the second attempt (p = 0.028), whereas during the third attempt, the youngest birds (30 weeks old) exhibited an increased duration of TI (p < 0.001). Additionally, we observed an interaction between age and the housing system on the second and third attempts (p = 0.003 and p = 0.010 respectively). This implies that the effects of age may exhibit variations in different housing environments or conditions, indicating a potential interplay or relationship between age and housing system factors. For a detailed analysis of the TI test attempts, including genotypes and their interactions, refer to the Supplementary material (Table S4).

### 3.4. Feather score and feet health assessment

The results of the feather score and the assessment of feet health are reported in Table 5. The housing system had a significant influence on both feather and FPD scores. Specifically, the laying hens housed in the enriched cage system exhibited poorer plumage and feet condition across all body parts compared to those in the free-range housing system (neck: p < 0.001; breast: p < 0.001; cloaca: p < 0.001; back: p < 0.001; wing: p < 0.001; tail: p < 0.001). Additionally, age was found to have an impact on the feather score, specifically in the back and cloaca regions (back: p = 0.012; cloaca: p = 0.03). No differences were found among the five genotypes in terms of feather and FPD scores.

**Table 5**
Absolute and relative frequencies of scores assigned to feather condition score and footpad dermatitis (FDP) of laying hens. Significant chi-square test results are highlighted in bold.

| | Genotype (G) | | | | | System (HS) | | Age (A) | |
|---|---|---|---|---|---|---|---|---|---|
| | BP | BPxS | LB | RM | RMxS | Free-range | Enriched cage | 30 weeks | 64 weeks |
| **Neck** | | | | | | | | | |
| 1 | 0(0%) | 0(0%) | 0(0%) | 0(0%) | 0(0%) | 0(0%) | 0(0%) | 0(0%) | 0(0%) |
| 2 | 0(0%) | 0(0%) | 0(0%) | 0(0%) | 0(0%) | 0(0%) | 0(0%) | 0(0%) | 0(0%) |
| 3 | 18(50%) | 20(55%) | 20(55%) | 19(53%) | 19(53%) | 6(7%) | 90(100%) | 48(53%) | 48(53%) |
| 4 | 18(50%) | 16(45%) | 16(45%) | 17(47%) | 17(47%) | 84(93%) | 0(0%) | 42(47%) | 42(47%) |
| | $X^2 = 0.31$; p-value = 0.98 | | | | | **$X^2 = 153.77$; p-value <0.001** | | $X^2=0$; p-value = 1 | |
| **Breast** | | | | | | | | | |
| 1 | 0(0%) | 0(0%) | 0(0%) | 0(0%) | 0(0%) | 0(0%) | 0(0%) | 0(0%) | 0(0%) |
| 2 | 0(0%) | 0(0%) | 0(0%) | 0(0%) | 0(0%) | 0(0%) | 0(0%) | 0(0%) | 0(0%) |
| 3 | 18(50%) | 19(53%) | 18(50%) | 18(50%) | 18(50%) | 1(2%) | 90(100%) | 46(51%) | 45(50%) |
| 4 | 18(50%) | 17(47%) | 18(50%) | 18(50%) | 18(50%) | 89(98%) | 0(0%) | 44(49%) | 45(50%) |
| | $X^2 = 0.08$; p-value = 0.99 | | | | | **$X^2 = 172.11$; p-value <0.001** | | $X^2 = 0$; p-value = 1 | |
| **Cloaca** | | | | | | | | | |
| 1 | 0(0%) | 0(0%) | 0(0%) | 0(0%) | 0(0%) | 0(0%) | 0(0%) | 0(0%) | 0(0%) |
| 2 | 2(6%) | 2(6%) | 1(3%) | 0(0%) | 1(3%) | 0(0%) | 6(7%) | 0(0%) | 6(7%) |
| 3 | 16(44%) | 17(47%) | 18(50%) | 19(53%) | 18(50%) | 17 | 84(93%) | 47(52%) | 40(44%) |
| 4 | 18(50%) | 17(47%) | 17(47%) | 17(47%) | 18(50%) | 18(50%) | 0(0%) | 43(48%) | 44(49%) |
| | $X^2 = 0.08$; p-value = 0.99 | | | | | **$X^2 = 168.41$; p-value <0.001** | | **$X^2=6.57$; p-value=0.03** | |
| **Back** | | | | | | | | | |
| 1 | 0(0%) | 0(0%) | 0(0%) | 0(0%) | 0(0%) | 0(0%) | 0(0%) | 0(0%) | 0(0%) |
| 2 | 0(0%) | 3(8%) | 1(3%) | 2(6%) | 2(6%) | 0(0%) | 8(9%) | 8(9%) | 0(0%) |
| 3 | 19(53%) | 16(44%) | 17(47%) | 16(44%) | 16(44%) | 2(2%) | 82(91%) | 38(42%) | 46(51%) |
| 4 | 17(47%) | 17(48%) | 18(50%) | 18(50%) | 18(50%) | 88(98%) | 0(0%) | 44(49%) | 44(49%) |
| | $X^2 = 3.72$; p-value = 0.88 | | | | | **$X^2 = 172.19$; p-value <0.001** | | **$X^2=8.76$; p-value=0.012** | |
| **Wing** | | | | | | | | | |
| 1 | 0(0%) | 0(0%) | 0(0%) | 0(0%) | 0(0%) | 0(0%) | 0(0%) | 0(0%) | 0(0%) |
| 2 | 0(0%) | 0(0%) | 0(0%) | 0(0%) | 0(0%) | 0(0%) | 0(0%) | 0(0%) | 0(0%) |
| 3 | 18(50%) | 19(53%) | 18(50%) | 19(53%) | 19(53%) | 3(3%) | 90(100%) | 47(52%) | 46(51%) |
| 4 | 18(50%) | 17(47%) | 18(50%) | 17(47%) | 17(47%) | 87(97%) | 0(0%) | 43(48%) | 44(49%) |
| | $X^2 = 0.133$; p-value = 0.9 | | | | | **$X^2 = 164.53$; p-value <0.001** | | $X^2 = 0$; p-value = 1 | |
| **Tail** | | | | | | | | | |
| 1 | 0(0%) | 0(0%) | 0(0%) | 0(0%) | 0(0%) | 0(0%) | 0(0%) | 0(0%) | 0(0%) |
| 2 | 5(14%) | 3(8%) | 2(6%) | 5(14%) | 6(17%) | 0(0%) | 21(23%) | 7(8%) | 14(16%) |
| 3 | 14(39%) | 15(42%) | 16(44%) | 14(39%) | 12(33%) | 2(2%) | 69(77%) | 39(53%) | 32(35%) |
| 4 | 17(47%) | 18(50%) | 18(50%) | 17(47%) | 18(50%) | 88(98%) | 0(0%) | 44(49%) | 44(49%) |
| | $X^2 = 3.26$; p-value = 0.91 | | | | | **$X^2 = 172.22$; p-value <0.001** | | $X^2 =3.02$; p-value = 0.22 | |
| **Footpad dermatitis** | | | | | | | | | |
| 0 | 20(55%) | 19(53%) | 19(53%) | 20(55%) | 16(44%) | 85(94%) | 9(10%) | 48(53%) | 46(51%) |
| 1 | 15(42%) | 15(42%) | 17(47%) | 15(42%) | 19(53%) | 5(6%) | 76(84%) | 42(47%) | 39(43%) |
| 2 | 1(3%) | 2(5%) | 0(0%) | 1(3%) | 1(3%) | 0(0%) | 5(6%) | 0(0%) | 5(6%) |
| | $X^2 = 3.36$; p-value = 0.91 | | | | | **$X^2 =128.68$; p-value <0.001** | | $X^2 =5.15$; p-value = 0.07 | |

LB: lohman brown; BP: bionda piemontese; BPxS: bionda piemontese x sasso; RM: robusta maculata; RMxS: robusta maculata x sasso.

## 4. Discussion

In summary, our findings underscore the profound impact of the housing system on the behaviour, plumage condition, and foot health of laying hens. The free-range system allows the hens to perform a broader range of behaviours, like movement, comfort, and social interactions. Additionally, hens in the free-range system exhibit improved plumage conditions and lower rates of footpad dermatitis, signifying a positive influence on their overall welfare as compared to the hens in the enriched cages. Furthermore, all genotypes demonstrate a similar behavioural pattern within the two housing systems. Notably, the age of the animals had minimal impact on the ethogram, plumage condition, or foot health, except for the results of the TI test.

In our study, we employed both a two-way analysis of variance (ANOVA) and the t-Stochastic Neighbor Embedding (t-SNE) technique. Employing these diverse analytical methods aims to provide a more comprehensive understanding of the complex behavioural patterns exhibited by the hens, especially when dealing with a larger set of behavioural variables such as the 16 behaviours scored in the present study. Our results demonstrate that the behavioural patterns exhibited by the hens are influenced by the housing system, with age and genotype having minimal effects. Despite expectations that the LB genotype might adapt more to cage systems and others to free-range systems, the observed behavioural responses across genotypes are quite similar. Indeed, despite behavioural differences among the various genotypes, all genotypes appear to demonstrate adaptability to both housing systems. However, for a thorough understanding of hen behaviour, a detailed study of individual behaviours, emphasizing the frequency and specific types, is essential.

The higher frequency of movement behaviours observed in the free-range system, including 'walking,' 'running,' and 'wing flapping,' can be attributed to the increased space and freedom available to the hens (Appleby, 2004). On the other hand, the lower frequency of 'running' behaviour exhibited by the LB genotype suggests that certain genotypes may be less inclined to engage in this specific behaviour. These results support the theory of energy conservation, suggesting that the higher kinetic activity of slow-growing animals reduces available body energy for productive performance(Castellini et al., 2012; Castellini and Bosco, 2017; Fanatico et al., 2007). Additionally, as highlighted by Schütz and Jensen (Schütz and Jensen, 2001), selection for high production rates can lead to modified behaviour. The free-range system provides a more spacious and natural environment, allowing the hens to engage in these movement activities more frequently. These results emphasize the impact of housing conditions, particularly the lack of space in the enriched cage system, on the expression of movement behaviours in laying hens. Providing hens with adequate space and environmental enrichment in enriched cages could potentially encourage more natural and active behaviours, contributing to their overall welfare (Xu et al., 2022).

In terms of the feeding category, it was observed that in the enriched cage system, laying hens spent more time engaging in feeding behaviour. This aligns with expectations, as the restricted environment of the enriched cage system encourages the birds to utilize the provided feeders for their feeding activities. On the other hand, in the free-range system, laying hens displayed a higher frequency of 'grass feeding and object pecking' behaviour. The presence of natural elements and objects in the free-range environment allows the hens to engage in foraging behaviours, such as pecking at grass and objects, aligning more closely with their natural feeding behaviour. This highlights the importance of providing hens with an extensive system rather than confining them to cages, significantly contributing to their welfare. Moreover, the extent to which animals can manifest their natural behaviours is deemed a cornerstone of animal welfare. This underscores the significance of creating environments conducive to such behaviours. Allowing animals to engage in behaviours typical of their natural habitats enhances their physical and psychological health, thus elevating overall standards of animal welfare (Colditz, 2023). This perspective underscores the necessity of comprehending and encouraging natural behaviours in assessing and implementing animal welfare protocols, encompassing the principles of the Five Freedoms and the Five Domains of Welfare (Brambell, 1965; Mellor et al., 2020).

The housing system significantly impacted resting behaviours, especially the 'crouching' behaviour, as indicated by our findings. Laying hens within the enriched cage system tended to display a higher frequency of 'crouching' behaviour, along with restricted movement. This behaviour may be associated with increased instances of footpad dermatitis and poorer feather quality, particularly in the breast area, as hens tend to rest more in that position (Göransson et al., 2023; Meluzzi and Sirri, 2009).

The higher occurrence of social behaviours such as 'pecking' and 'fighting' in the enriched cage system can be attributed to several factors. Firstly, the lack of environmental stimuli and limited space in enriched cages may contribute to increased aggression among the confined animals (Nicol et al., 1999). This aggressive behaviour may have implications for the plumage status of the hens, particularly in areas such as the neck, tail, and wings. The higher stocking density in cage systems compared to free-range systems can further escalate social interactions and competition for resources. This is also confirmed by a greater amount of time lying down in the enriched cage system, where the animals seem to be less active and interested in their surroundings, reducing object pecking behaviour (Koelkebeck et al., 1987). Additionally, the cage flooring itself can contribute to the development of footpad dermatitis.

Regarding genotype differences, our analysis revealed that the LB genotype exhibited less engagement in social behaviours, specifically 'allo-preening' and 'fighting', compared to the other genotypes. This suggests a potential genetic influence on social behaviour tendencies in laying hens. The LB genotype may have inherent traits or characteristics that contribute to a decreased inclination for social interactions, resulting in less time spent on allo-preening and engaging in fights (Wurtz et al., 2022). Furthermore, it is conceivable that social behaviour has been influenced by selection in LB, potentially aimed at preventing injurious pecking behaviour within larger flocks.

The higher frequency of stretching observed in the enriched cage system can be interpreted as a compensatory behaviour for the lack of movement. Chickens often engage in stretching activities involving their wings and legs when they experience muscle stiffness or joint pain, potentially resulting from inadequate physical exercise (Albentosa and Cooper, 2004; Engel et al., 2019; Hughes and Duncan, 1988; Nicol, 1987). This behaviour may be interpreted as a response to discomfort, indicating an attempt to alleviate potential muscle and joint tension or discomfort experienced by the chickens.

The influence of the housing system, genotype, and their interaction on comfort behaviours, specifically 'sand bath' and 'self-preening', is evident from our analysis. The LB genotype exhibited the lowest percentage of these behaviours, indicating potential differences in their expression across genotypes. However, it is important to interpret the results of self-preening behaviour cautiously. While self-preening is a natural behaviour for chickens to maintain feather condition and cleanliness, excessive self-preening can be indicative of underlying anxiety or stress. In environments where animals are unable to express freely or are subjected to confined spaces, they may engage in excessive comfort behaviours as a coping mechanism, which can be described as stereotypical behaviour (Broom, 1983).

Moreover, the evident disparity between the two housing systems, as evidenced by the analysis of FPD and feather condition scores, underscores how the free-range system better fulfills the birds' requirements. For instance, it offers a superior flooring that mitigates the occurrence of FPD and enables chickens to express their natural behaviours more freely, thereby reducing the likelihood of feather pecking. In fact, all the animals housed in the free-range system didn't present any FPD nor feather damage contrary to what was found in the enriched cages system, as previously reported also by other authors (Erensoy et al., 2021; Rørvang et al., 2019; Wei et al., 2020). The lack of differences in TI results showed how the examined genotypes did not exhibit distinct variations in their responses to the test. Contrary, a consistent trend towards greater adrenocortical activation was reported in high-fear hens, which showed long TI reactions when compared to low-fear counterparts (Beuving et al., 1989). Interestingly, hen's age did not affect general behaviours, but it did influence the response in the TI test. TI in chickens serves as a fear indicator, with susceptibility changing during development and being influenced by various factors like exposure to threatening stimuli (Tiemann et al., 2023). However, the effectiveness of TI as a fear indicator, especially concerning age, is debated. Studies suggest that TI duration in chickens can be modulated by environmental and individual factors, with age impacting susceptibility to induction procedures. Additionally, genetic predispositions inherited in different chicken breeds lead to substantial variation in fear responses, indicating that TI might not be universally effective across all ages and breeds (Carli and Farabollini, 2022).

These results highlight the complex nature of stress response in laying hens, indicating that age can play a role in physiological reactions to the environment. Our findings underscore the importance of continually evaluating and refining housing systems to ensure the welfare of laying hens. Efforts should be made to strike a balance between productivity and animal welfare, considering the natural behavioural needs and health requirements of the birds.

## 5. Conclusions

In conclusion, our research has revealed differences in the adaptability of different chicken genotypes to various farming systems. While it may initially seem that all genotypes adapt well, a deeper analysis revealed that the enriched cage system can restrict natural behaviours and have adverse effects on feather and leg health. These findings underscore the importance of carefully assessing the specific impacts of farming systems on hens to develop more sustainable poultry practices that prioritize animal welfare. Analyzing chicken behaviour enables early problem detection and implementation of preventive measures. Prioritizing animal welfare by farmers, the poultry industry, and consumers is essential for advocating ethical practices and alternative farming systems, ensuring humane treatment of birds and the industry's long-term sustainability.

## Institutional Review Board Statement

The experimental protocol was approved by the Bioethical Committee of the University of Bologna (Prot. ID: 1102/2019).

## Funding

This research was funded by the Ministero dell'Istruzione, dell'Università e della Ricerca [PRIN National Grant 2017 – Prot. 2017S229WC].

## CRediT authorship contribution statement

**Martina Tarantola:** Investigation, Funding acquisition, Conceptualization. **Patrizia Ponzio:** Investigation, Data curation, Conceptualization. **Cecilia Mugnai:** Writing – original draft, Methodology, Investigation, Funding acquisition, Data curation, Conceptualization. **Edoardo Fiorilla:** Writing – review & editing, Writing – original draft, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Laura Ozella:** Writing – review & editing, Writing – original draft, Visualization, Methodology, Formal analysis, Data curation. **Federico Sirri:** Methodology, Investigation, Data curation, Conceptualization. **Marco Zampiga:** Methodology, Investigation, Conceptualization. **Raffaela Piscitelli:** Methodology, Investigation, Data curation, Conceptualization.

## Declaration of Competing Interest

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

## Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.applanim.2024.106247.

## References

Albentosa, M.J., Cooper, J.J., 2004. Effects of cage height and stocking density on the frequency of comfort behaviours performed by laying hens housed in furnished cages. Anim. Welf. 13, 419–424 https://doi.org/DOI: 10.1017/S0962728600028670.

Appleby, M.C., 2004. What causes crowding? Effects of space, facilities and group size on behavior, with particular reference to furnished cages for hens. Anim. Welf. 13, 313–320. https://doi.org/10.1017/S0962728600028426.

Besbes, B., Gibson, J.P., 1999. Genetic variation of egg production traits in purebred and crossbred laying hens. Anim. Sci. 68, 433–439 https://doi.org/DOI: 10.1017/S135772980005044X.

Beuving, G., Jones, R.B., Blokhuis, H.J., 1989. Adrenocortical and heterophil/lymphocyte responses to challenge in hens showing short or long tonic immobility reactions. Br. Poult. Sci. 30, 175–184. https://doi.org/10.1080/00071668908417135.

Biasato, I., Oddon, S.B., Chemello, G., Gariglio, M., Fiorilla, E., Dabbou, S., Pipan, M., Dekleva, D., Macchi, E., Gasco, L., Schiavone, A., 2022. Welfare implications for broiler chickens reared in an insect larvae-enriched environment: focus on bird behaviour, plumage status, leg health, and excreta corticosterone. Front. Physiol. 1–16. https://doi.org/10.3389/fphys.2022.930158.

Brambell, F.W.R., 1965. Report of the Technical Committee to Enquire Into the Welfare of Animals Kept Under Intensive Livestock Husbandry Systems. Fisheries (Bethesda) 85.

Broom, D.M., 1983. In: Smidt, D. (Ed.), Stereotypies as Animal Welfare Indicators BT - Indicators Relevant to Farm Animal Welfare: A Seminar in the CEC Programme of Coordination of Research on Animal Welfare, organized by Dr. D. Smidt, and held in Mariensee, 9-10 November 1982. Springer Netherlands, Dordrecht, pp. 81–87. https://doi.org/10.1007/978-94-009-6738-0_11.

Bruijnis, M.R.N., Blok, V., Stassen, E.N., Gremmen, H.G.J., 2015. Moral "lock-in" in responsible innovation: the ethical and social aspects of killing day-old chicks and its alternatives. J. Agric. Environ. Ethics 28, 939–960. https://doi.org/10.1007/s10806-015-9566-7.

Campbell, D.L.M., Dickson, E.J., Lee, C., 2019. Application of open field, tonic immobility, and attention bias tests to hens with different ranging patterns. PeerJ 7, e8122. https://doi.org/10.7717/peerj.8122.

Campbell, D.L.M., Dyall, T.R., Downing, J.A., Cohen-Barnhouse, A.M., Lee, C., 2020. Rearing enrichments affected ranging behavior in free-range laying hens. Front. Vet. Sci. 7 https://doi.org/10.3389/fvets.2020.00446.

Campbell, D.L.M., Bari, M.S., Rault, J.-L., 2021. Free-range egg production: its implications for hen welfare. Anim. Prod. Sci. 61, 848–855.

Carli, G., Farabollini, F., 2022. Chapter 5 - Environmental, ecological and methodological factors of Tonic Immobility (TI) modulation. In: Carli, G., Farabollini, F. (Eds.), Progress in Brain Research. Elsevier, pp. 101–132. https://doi.org/10.1016/bs.pbr.2022.02.005.

Carvalho, C.L., Andretta, I., Galli, G.M., Martins, G.B., Camargo, N. de O.T., Stefanello, T. B., Melchior, R., da Silva, M.K., 2022. Dietary supplementation with β-mannanase and probiotics as a strategy to improve laying hen's welfare. Front. Vet. Sci.

Castellini, C., Bosco, A., 2017. Animal welfare and poultry meat in alternative production systems (and ethics of poultry meat production). Poult. Qual. Eval. 335–357. https://doi.org/10.1016/B978-0-08-100763-1.00014-3.

Castellini, C., Boggia, A., Cortina, C., Bosco, A., Paolotti, L., Novelli, E., C, M., 2012. A multicriteria approach for measuring the sustainability of different poultry production systems. J. Clean. Prod. 37, 192–201. https://doi.org/10.1016/j.jclepro.2012.07.006.

Colditz, I.G., 2023. A biological integrity framework for describing animal welfare and wellbeing. Anim. Prod. Sci. 63, 423–440.

van der Maaten, L., Hinton, G., 2008. Vualizing data using t-SNE. J. Mach. Learn. Res. 9, 2579–2605.

Engel, J.M., Widowski, T.M., Tilbrook, A.J., Butler, K.L., Hemsworth, P.H., 2019. The effects of floor space and nest box access on the physiology and behavior of caged laying hens. Poult. Sci. 98, 533–547. https://doi.org/10.3382/ps/pey378.

Erensoy, K., Sarıca, M., Akif Boz, M., Uçar, A., 2021. Health welfare of laying hens reared in cage and non-cage systems. Int. J. Poult. Ornam. Birds Sci. Technol. 2, 30–35.

Fanatico, A.C., Pillai, P.B., Emmert, J.L., Owens, C.M., 2007. Meat quality of slow- and fast-growing chicken genotypes fed low-nutrient or standard diets and raised indoors or with outdoor access. Poult. Sci. 86, 2245–2255. https://doi.org/10.1093/ps/86.10.2245.

Ferrante, V., Marelli, S.P., Pignattelli, P., Baroli, D., Cavalchini, L.G., 2005. Performance and reactivity in three Italian chicken breeds for organic production.

Fiorilla, E., Birolo, M., Ala, U., Xiccato, G., Trocino, A., Schiavone, A., Mugnai, C., 2023. Productive performances of slow-growing chicken breeds and their crosses with a commercial strain in conventional and free-range farming systems. Animals. https://doi.org/10.3390/ani13152540.

Fiorilla, E., Gariglio, M., Martinez-Miro, S., Rosique, C., Madrid, J., Montalban, A., Biasato, I., Bongiorno, V., Cappone, E.E., Soglia, D., Schiavone, A., 2024. Improving sustainability in autochthonous slow-growing chicken farming: exploring new frontiers through the use of alternative dietary proteins. J. Clean. Prod. 434, 140041 https://doi.org/10.1016/j.jclepro.2023.140041.

Friard, O., Gamba, M., 2016. BORIS: a free, versatile open-source event-logging software for video/audio coding and live observations. Methods Ecol. Evol. 7, 1325–1330. https://doi.org/10.1111/2041-210X.12584.

Göransson, L., Abeyesinghe, S., Yngvesson, J., Gunnarsson, S., 2023. How are they really doing? Animal welfare on organic laying hen farms in terms of health and behaviour. Br. Poult. Sci. Null. Null. https://doi.org/10.1080/00071668.2023.2241829.

Grebey, T.C., Ali, A.B.A., Swanson, J.C., Widowski, T.M., Siegford, J.M., 2020. Dust bathing in laying hens: strain, proximity to, and number of conspecifics matter. Poult. Sci. 99, 4103–4112. https://doi.org/10.1016/j.psj.2020.04.032.

Hughes, B.O., Duncan, I.J.H., 1988. The notion of ethological 'need', models of motivation and animal welfare. Anim. Behav. 36, 1696–1707. https://doi.org/10.1016/S0003-3472(88)80110-6.

Hurnik, F., Webster, A.B., 1990. An ethogram of White Leghorn-Type hens in battery cages. Can. J. Anim. Sci. 760, 751–760.

Hendrix Genetics and Sasso Management Guide, 2022 Sasso Management Guide, 2022. https://europe.sassopoultry.com/en/resources/management-guides/.

Janczak, A.M., Riber, A.B., 2015. Review of rearing-related factors affecting the welfare of laying hens. Poult. Sci. 94, 1454–1469. https://doi.org/10.3382/ps/pev123.

Jhetam, S., Buchynski, K., Shynkaruk, T., Schwean-Lardner, K., 2022. Evaluating the effects of stocking density on the behavior, health, and welfare of Turkey hens to 11 weeks of age. Poult. Sci. 101, 101956 https://doi.org/10.1016/j.psj.2022.101956.

Kayla, A., Nigel, J., Kristie, E., 2023. Hierarchical structure and feeding behaviours of free-range hens (Gallus gallus domesticus). Int. J. Comp. Psychol. 0–12.

Kenny, E., Birkhead, T.R., Green, J.P., 2017. Allopreening in birds is associated with parental cooperation over offspring care and stable pair bonds across years. Behav. Ecol. 28, 1142–1148. https://doi.org/10.1093/beheco/arx078.

Koelkebeck, K.W., Amoss, M.S., Cain, J.R., 1987. Production, physiological, and behavioral responses of laying hens in different management environments. Poult. Sci. 66, 397–407. https://doi.org/10.3382/ps.0660397.

Lai, P.W., Liang, J.B., Hsia, L.C., Loh, T.C., Ho, Y.W., 2010. Effects of varying dietary zinc levels and environmental temperatures on the growth performance, feathering score and feather mineral concentrations of broiler chicks. Asian-Austral J. Anim. Sci. 23, 937–945. https://doi.org/10.5713/ajas.2010.90495.

Lay, D.C., Fulton, R.M., Hester, P.Y., Karcher, D.M., Kjaer, J.B., Mench, J.A., Mullens, B. A., Newberry, R.C., Nicol, C.J., O'Sullivan, N.P., Porter, R.E., 2011. Hen welfare in different housing systems. Poult. Sci. 90 (1), 278–294. https://doi.org/10.3382/ps.2010-00962.

Liu, Z., Torrey, S., Newberry, R.C., Widowski, T., 2020. Play behaviour reduced by environmental enrichment in fast-growing broiler chickens. Appl. Anim. Behav. Sci. 232, 105098 https://doi.org/10.1016/j.applanim.2020.105098.

Mancinelli, A.C., Menchetti, L., Birolo, M., Bittante, G., Chiattelli, D., Castellini, C., 2023. Crossbreeding to improve local chicken breeds: predicting growth performance of the crosses using the Gompertz model and estimated heterosis. Poult. Sci. 102, 102783 https://doi.org/10.1016/j.psj.2023.102783.

McCowan, B., Schrader, J., DiLorenzo, A.M., Cardona, C., Klingborg, D., 2006. Effects of induced molting on the well-being of egg-laying hens. J. Appl. Anim. Welf. Sci. 9, 9–23. https://doi.org/10.1207/s15327604jaws0901_2.

Mellor, D.J., Beausoleil, N.J., Littlewood, K.E., McLean, A.N., McGreevy, P.D., Jones, B., Wilkins, C., 2020. The 2020 five domains model: including human–animal interactions in assessments of animal welfare. Animals 10. https://doi.org/10.3390/ani10101870.

Meluzzi, A., Sirri, F., 2009. Welfare of broiler chickens. Ital. J. Anim. Sci. 8, 161–173. https://doi.org/10.4081/ijas.2009.s1.161.

Nicol, C.J., 1987. Behavioural responses of laying hens following a period of spatial restriction. Anim. Behav. 35, 1709–1719. https://doi.org/10.1016/S0003-3472(87)80063-5.

Nicol, C.J., Gregory, N.G., Knowles, T.G., Parkman, I.D., Wilkins, L.J., 1999. Differential effects of increased stocking density, mediated by increased flock size, on feather pecking and aggression in laying hens. Appl. Anim. Behav. Sci. 65, 137–152. https://doi.org/10.1016/S0168-1591(99)00057-X.

Rieke, L., Spindler, B., Zylka, I., Kemper, N., Giersberg, M.F., 2021. Pecking behavior in conventional layer hybrids and dual-purpose hens throughout the laying period. Front. Vet. Sci. 8, 1–10. https://doi.org/10.3389/fvets.2021.660400.

Rodenburg, T.B., Tuyttens, F.A.M., de Reu, K., Herman, L., Zoons, J., Sonck, B., 2008. Welfare assessment of laying hens in furnished cages and non-cage systems: an on-farm comparison. Anim. Welf. 17, 363–373 https://doi.org/DOI: 10.1017/S096272860002786X.

Rørvang, M.V., Hinrichsen, L.K., Riber, A.B., 2019. Welfare of layers housed in small furnished cages on Danish commercial farms: the condition of keel bone, feet, plumage and skin. Br. Poult. Sci. 60, 1–7. https://doi.org/10.1080/00071668.2018.1533632.

Rushen, J., Butterworth, A., Swanson, J., 2011. Animal behavior and well-being symposium:Farm animal welfare assurance: Science and application. J. Anim. Sci. 89, 1219–1228. https://doi.org/10.2527/jas.2010-3589.

Schütz, K.E., Jensen, P., 2001. Effects of resource allocation on behavioural strategies: a comparison of red junglefowl (Gallus gallus) and two domesticated breeds of poultry. Ethology 107, 753–765. https://doi.org/10.1046/j.1439-0310.2001.00703.x.

Stefanetti, V., Mancinelli, A.C., Pascucci, L., Menchetti, L., Castellini, C., Mugnai, C., Fiorilla, E., Miniscalco, B., Chiattelli, D., Franciosini, M.P., Proietti, P.C., 2023. Effect of rearing systems on immune status, stress parameters, intestinal morphology, and mortality in conventional and local chicken breeds. Poult. Sci. 102, 103110 https://doi.org/10.1016/j.psj.2023.103110.

Tauson, R., Kjaer, J., Maria, G., Cepero, R., Maria Levrino, G., Cepero, R., 2006. Applied scoring of integument and health in laying hens. Anim. Sci. Pap. Rep. 23, 153–159.

Tiemann, I., Becker, S., Fournier, J., Damiran, D., Buescher, W., Hillemacher, S., 2023. Differences among domestic chicken breeds in tonic immobility responses as a measure of fearfulness. PeerJ 11, e14703. https://doi.org/10.7717/peerj.14703.

Vale, P., Gibbs, H., Vale, R., Christie, M., Florence, E., Munger, J., Sabaini, D., 2019. The expansion of intensive beef farming to the Brazilian Amazon. Glob. Environ. Change 57, 101922. https://doi.org/10.1016/j.gloenvcha.2019.05.006.

Veldkamp, T., Niekerk, T.G.C.M., 2019. Live black soldier fly larvae (Hermetia illucens) for Turkey poults. J. Insects Food Feed 5, 1–12. https://doi.org/10.3920/JIFF2018.0031.

Webster, A.B., 2000. Behavior of white leghorn laying hens after withdrawal of feed. Poult. Sci. 79, 192–200. https://doi.org/10.1093/ps/79.2.192.

Wei, H., Bi, Y., Xin, H., Pan, L., Liu, R., Li, X., Li, J., Zhang, R., Bao, J., 2020. Keel fracture changed the behavior and reduced the welfare, production performance, and egg quality in laying hens housed individually in furnished cages. Poult. Sci. 99, 3334–3342. https://doi.org/10.1016/j.psj.2020.04.001.

Wurtz, K.E., Thodberg, K., Berenjian, A., Foldager, L., Tahamtani, F.M., Riber, A.B., 2022. Commercial layer hybrids kept under organic conditions: a comparison of range use, welfare, and egg production in two layer strains. Poult. Sci. 101, 102005 https://doi.org/10.1016/j.psj.2022.102005.

Xu, D., Shu, G., Liu, Y., Qin, P., Zheng, Y., Tian, Y., Zhao, X., Du, X., 2022. Farm environmental enrichments improve the welfare of layer chicks and pullets: a comprehensive review. Animals 12. https://doi.org/10.3390/ani12192610.