

Behavioral and Physiological Welfare Responses of Rhode Island Red Hens Reared in Individual Cage, Colony Cage and Deep-Litter Systems

Abhay Kumar

abhay02k3@gmail.com

Bihar Animal Sciences University

R. R. K. Sinha

Bihar Animal Sciences University

S. P. Sahu

Bihar Animal Sciences University

K. Kumar

Bihar Animal Sciences University

A.K. Kumar Singh

Banaras Hindu University - South Campus: Banaras Hindu University - Rajiv Gandhi South Campus

A. Bharti

Bihar Animal Sciences University

Research Article

Keywords: Rhode Island Red, housing system, welfare indicators, tonic immobility, heterophil-to-lymphocyte ratio, corticosterone, laying hens

Posted Date: June 24th, 2026

DOI: <https://doi.org/10.21203/rs.3.rs-9950223/v1>

License:   This work is licensed under a Creative Commons Attribution 4.0 International License.

[Read Full License](#)

Abstract

The present experiment was conducted to compare three housing systems Individual cages, Colony cages and Deep litter on welfare, behaviour, and physiological traits in Rhode Island Red laying hens under tropical conditions. A total of 120 birds (40 per treatment) were housed from 16 to 40 weeks of age. Feather condition, foot lesions, keel bone deformities, and gait performance did not differ significantly among housing systems ($P > 0.05$). Claw length was shorter in deep litter birds (57.4 ± 0.6 mm) than in individual cages (61.8 ± 1.0 mm; $P < 0.05$). Tonic immobility duration, a fear response indicator, was lower in deep litter (64.6 ± 10.7 s) and colony cage birds (72.1 ± 10.2 s) compared with individually caged birds (171.3 ± 24.1 s; $P < 0.05$).

At 40 weeks, deep litter birds showed higher triiodothyronine (T3) concentrations (148 vs. 124–127 ng/dL; $P < 0.05$) and a lower heterophil-to-lymphocyte (H/L) ratio (0.52 vs. 0.60–0.61; $P < 0.05$) compared with caged birds. However, corticosterone concentrations were also higher in deep litter birds (2.54 vs. 1.94–2.19 ng/mL; $P < 0.05$).

Welfare indicators did not respond uniformly across housing systems. Deep litter housing was associated with shorter claw length, reduced fear responses, and lower H/L ratios, but also with elevated corticosterone-highlighting the multidimensional nature of welfare assessment.

Most biochemical and physical welfare indicators remained unaffected under the management conditions of this study. Our results suggest that housing systems are associated with differences in specific welfare indicators. Further studies employing multiple independent replicates of each housing system, larger sample sizes, and longer production cycles are required to confirm these findings and support robust recommendations for poultry welfare management in tropical production systems.

INTRODUCTION

Poultry welfare is receiving increasing global attention as an important component of sustainable livestock production, influencing animal health, productivity, product quality, and consumer acceptance. In India, where the poultry sector plays a significant role in food security, nutritional supply, and rural livelihoods, there is growing interest in production systems that balance economic efficiency with animal welfare. Housing environment is one of the major factors affecting poultry welfare because it directly influences behavioural expression, physiological status, health, and adaptation to environmental challenges (Umber Rauf et al., 2024). Among the various production systems, individual cages, colony cages, and deep litter systems differ considerably in the degree of space allowance, environmental complexity, and opportunities for natural behaviours. Cage systems are widely adopted because of their advantages in management, feed efficiency, and space utilization. However, restricted movement and limited behavioural opportunities may adversely affect welfare by increasing stress, impairing skeletal development, and reducing the expression of natural behaviours (Sonkamble, 2020). Conversely, deep litter systems provide greater opportunities for walking, scratching, dust bathing, and social interaction, which may support behavioural well-being and musculoskeletal health. Nevertheless, the welfare

benefits of deep litter housing can be influenced by management practices, stocking density, litter quality, and environmental hygiene, potentially increasing risks associated with pathogen exposure and social competition (Fiorilla et al., 2024). Existing comparative research suggests that cage systems may result in better feed conversion and egg quality, but often at the cost of reduced body condition and feather quality—hallmarks of suboptimal welfare (Pires et al., 2021) and reduced egg quality (Baylan et al., 2024) (Susilo et al., 2024). Conversely, non-cage systems have been linked with improved bone quality, reduced keel bone damage, and greater behavioural expression, although their effects on health and productivity have not been consistent across studies (Kraus et al., 2021) (Kraus et al., 2022). These inconsistencies suggest that housing-related welfare outcomes are influenced not only by the production system itself but also by local environmental and management conditions. Assessment of poultry welfare requires the integration of behavioural, physical, and physiological indicators. Physiological variables such as corticosterone, glucose, total protein, alanine aminotransferase (ALT), and heterophil-to-lymphocyte (H/L) ratio provide valuable information on stress response, metabolic status, and immune function. Environmental conditions, including stocking density and microclimate, can substantially affect these indicators (Eid et al., 2025). Heat stress, a common challenge in tropical and subtropical regions, has been shown to alter leukocyte profiles and H/L ratios, reflecting changes in immune competence and physiological adaptation (Mashaly et al., 2004); (Erek and Matur, 2024) ; (Sabo et al., 2025). Behavioural indicators offer additional insight into welfare status. Tonic immobility (TI), a widely accepted measure of fearfulness in poultry, generally decreases in birds maintained in enriched or cage-free environments compared with conventional cages (Rentsch et al., 2023; Campbell et al., 2022). However, some studies have reported no significant differences among housing systems, indicating that breed, management, and environmental conditions may influence behavioural responses (Yilmaz Dikmen et al., 2016) found no significant differences in TI responses across housing systems, suggesting that outcomes may vary depending on management practices and environmental factors. Similarly, feather condition is considered an important welfare indicator because it reflects health status, social interactions, and environmental suitability. Improved plumage quality has frequently been reported in free-range and deep litter systems (Sherwin et al., 2010), (Blatchford et al., 2016), (Pichová et al., 2017), (Hüttner et al., 2023). In contrast, (Yilmaz Dikmen et al., 2016) found slightly increased neck feather loss in conventional cages relative to furnished cages, although the overall feather score did not differ significantly. Despite growing international research on poultry welfare, information comparing behavioural, physical, and physiological welfare indicators under different housing systems remains limited under Indian production conditions, particularly in eastern India where climatic variability, management practices, and resource availability may influence bird responses. Furthermore, welfare assessments in RIR hens, an important breed for backyard and alternative production systems, are relatively scarce. Therefore, the present study was undertaken to compare the effects of individual cage, colony cage, and deep litter housing systems on selected physical, behavioural, and physiological welfare indicators in Rhode Island Red laying hens reared under tropical eastern Indian conditions.

MATERIALS AND METHODS

Experimental Birds, Housing and Management

The experiment was conducted at the Poultry Research and Training Centre (PR&TC), Bihar Animal Sciences University (BASU), Patna, India, from November 2023 to May 2024. A total of 120 Rhode Island Red (RIR) pullets with an average body weight of 1.01 ± 0.04 kg at 16 weeks of age were randomly assigned to one of three housing systems: individual cages (IC), colony cages (CC), and deep litter system (DLS), with 40 birds allocated to each treatment. Birds were maintained in their respective housing systems until 40 weeks of age. The individual cage system consisted of cages measuring 60 cm × 32 cm × 53 cm (length × width × height), housing one bird per cage. Cage floors were constructed from welded wire mesh (3.5 cm × 1.0 cm) and slightly sloped to facilitate egg collection. Each cage was equipped with a linear feeder and a water trough, providing unrestricted access to feed and water. The colony cage system comprised cages measuring 191 cm × 60 cm × 53 cm (length × width × height), accommodating five birds per cage. Colony cages were arranged in a three-tier configuration and provided environmental enrichments including perches, nest boxes, scratching pads, and designated dust-bathing areas. Feed and water delivery systems were similar to those used in the individual cages. Cage design, flooring material, feeder type, and general management practices were kept consistent between the two cage systems to minimize housing-related confounding factors. Manure was removed twice weekly to maintain hygiene and environmental quality. The deep litter system consisted of a floor pen (10 ft × 10 ft) bedded with a 10-12 cm layer of clean, dry wood shavings. Prior to use, the litter was treated with slaked lime to reduce microbial contamination. Litter was regularly stirred and replenished when required to maintain suitable moisture levels and hygienic conditions. Birds housed in the deep litter system were provided with the same enrichments available in colony cages, including perches, nest boxes, scratching pads, and dust-bathing facilities. Feed was supplied through trough feeders and water through plastic drinkers, which were cleaned daily. All birds were managed under uniform husbandry conditions throughout the study. The average temperature (22-35°C), relative humidity (55 to 75%) and photoperiod (16-18 hours of light per day) are respectively for the study period. A standard layer diet formulated to meet the nutrient requirements of growing and laying Rhode Island Red hens was offered ad libitum, and fresh drinking water was continuously available. Routine health care and management practices were followed across all treatment groups. Environmental conditions within the poultry house were monitored regularly and maintained within acceptable management limits to minimize the influence of non-treatment-related stressors.

The effects of housing system on bird welfare were evaluated using a combination of physical, behavioural, and physiological indicators. Physical assessments included feather condition, foot lesion score, keel bone deformity, gait performance, and claw length. Behavioural welfare was assessed using tonic immobility response, while physiological evaluation included selected serum biochemical parameters, thyroid hormones, heterophil-to-lymphocyte ratio, and corticosterone concentration (Bhadauria et al., 2019). The research proposal was approved by IAEC with reference number-IAEC/BVC/2024/31.

Clinical welfare indicators

The assessment area was spacious, well-lit, and free from obstacles. Birds were gently caught handled carefully and calmed before being placed on the ground to walk freely. The assessment focused on balance, coordination, posture, and speed as the birds moved around the area

Feather condition: Ten birds from each housing system were randomly selected at 20, 28, 32, 36, and 40 weeks of age to assess feather condition at seven body areas (head, neck, back, wings, tail, abdomen, and breast) using a five-point scoring system (Abrahamsson et al., 1996) and evaluated based on the scoring system: 1 for full feather cover, 2 for worn feathers, 3 for small bare patches (<30 mm), 4 for large bare patches (>30 mm), and 5 for no feathers in most areas.

Foot Lesion: Ten birds from each housing system were randomly selected for foot lesion assessment using the scoring system from (Nicol et al., 2006). The assessment area was clean, dry, and suitable for handling.

Keel Bone Deformity: Ten birds from each housing system were randomly selected for keel bone deformity assessment using the (Wilkins et al., 2011) scoring system.

Gait Performance: At 20 weeks of age, ten birds from each housing system were randomly selected for gait performance testing, evaluated on a scale from 0 to 5 based on (Kestin et al., 1992).

Claw Length: To measure claw length (in mm), ten birds from each housing system were randomly selected. The assessment area was clean, well-lit, and suitable for handling. Using a ruler, the middle toe of each bird's right foot was measured from the base to the tip. Birds were gently restrained to ensure accurate and consistent measurements without causing stress or discomfort.

Tonic Immobility: Tonic immobility (TI) was induced following the method of (Jones and Faure, 1981) in a quiet, controlled space. At 20 weeks, ten birds from each housing system were randomly selected. If a bird righted itself in under 10 seconds, the induction was considered a failure. After three failed attempts, the bird was scored 0 seconds. If no righting response occurred within 10 minutes, a maximum score of 600 seconds was given (Campo et al., 2008). To induce TI, each bird was gently caught, restrained on its back, and stroked on the chest or abdomen. The duration of TI was recorded with a stopwatch once the bird exhibited signs of immobility, such as remaining motionless with closed eyes. Afterward, the bird was released to recover in a comfortable environment.

Plasma hormone profiles and biochemical markers:

Blood samples were collected from ten birds (randomly selected) in each group at 16 and 40 weeks of age. Two mL of blood were drawn from the wing vein, allowed to clot at room temperature for 30 minutes, and then centrifuged at 3000 RPM for 10 minutes. The serum was separated and analysed for corticosterone, T3, T4, ALT, AST, glucose, protein profile and lipid profile using commercial kits. Serum corticosterone, T3, T4 were analysed using ELISA kits (Bioassay Technology Laboratory, BT LAB, China). ALT, AST, glucose, protein and lipid profiles were measured using an automatic biochemical analyzer (Erba Mannheim Chemist 5X) with ERBA commercial diagnostic kits.

Note-For each assessment time point, ten birds per housing system were randomly selected from the 40 birds in each group. The same birds were not necessarily used across time points. Further, Since welfare indicators were assessed at multiple ages, each time point was analysed separately to compare housing systems within a given age. A repeated-measures approach was not used because different birds were sampled at each age.

Statistical Analysis

The study was conducted using a completely randomized design (CRD) involving three housing systems: individual cage (IC), colony cage (CC), and deep litter system (DLS). A total of 120 Rhode Island Red hens were randomly allocated to the three treatments, with 40 birds assigned to each housing system. Welfare assessments were conducted at predefined intervals between 16 and 40 weeks of age to evaluate the effects of housing environment on physical, behavioural, and physiological welfare indicators. Descriptive statistics were calculated for all measured variables and are presented as mean $\pm$ standard error of the mean (SEM). Prior to analysis, data were examined for normality using the Shapiro–Wilk test and for homogeneity of variance using Levene’s test. For continuous variables that satisfied assumptions of normality and homogeneity of variance, including claw length and serum biochemical parameters, differences among housing systems were analysed using one-way analysis of variance (ANOVA). When a significant treatment effect was detected, means were separated using Tukey’s honestly significant difference (HSD) test. Variables measured repeatedly across different ages were analysed separately at each sampling point to evaluate housing system effects within a given age. For ordinal or non-normally distributed data, including feather condition score, foot lesion score, keel bone deformity score, gait score, and tonic immobility responses, the non-parametric Kruskal-Wallis H test was employed. When significant differences were detected, pairwise comparisons were performed using Dunn’s procedure with appropriate adjustment for multiple comparisons. Statistical analyses were performed using SPSS Statistics Version 20.0 (IBM Corp., Armonk, NY, USA). Graphs and figures were prepared using Microsoft Excel. Differences were considered statistically significant at $P < 0.05$, while values between $P \geq 0.05$ and $P < 0.10$ were interpreted as indicating a statistical tendency.

RESULT AND DISCUSSION

Since feather condition, foot lesion score, keel bone deformity score, and gait score were ordinal variables, data were analysed using the Kruskal-Wallis test and are presented as mean rank-derived scores (mean $\pm$ SEM)

Feather condition scores of birds at different ages during experimental period:

Feather condition scores of RIR hens under different housing systems are presented in Table 1. Up to 28 weeks of age, all birds maintained excellent plumage condition with a uniform score of 1, indicating the absence of feather damage across treatments. From 32 weeks onward, a gradual increase in feather scores was observed in all groups, suggesting some deterioration in plumage condition with advancing age. However, housing system did not significantly influence feather condition at any observation period.

Feather scores were comparable among individual cages, colony cages, and deep litter systems at 32 weeks ($P = 0.420$), 36 weeks ($P = 0.640$), and 40 weeks of age ($P = 0.882$). Although birds maintained on deep litter tended to exhibit numerically lower feather scores at later ages, these differences were not statistically significant. Overall, the results indicate that feather condition remained largely unaffected by housing system under the management conditions of the present study.

Table 1. Feather condition scores of birds at different ages across housing systems

Age (weeks)	Individual Cage	Colony Cage	Deep Litter	H (df=2)	P value
32	1.0 (1.0-2.0)	1.0 (1.0-2.0)	1.0 (1.0-2.0)	0.81	0.420
36	2.0 (1.0-2.0)	2.0 (1.0-3.0)	1.5 (1.0-2.0)	0.89	0.640
40	2.0 (1.0-3.0)	2.0 (1.0-3.0)	1.5 (1.0-2.0)	0.25	0.880

Feather condition scores: 1 = full feather cover; 2 = worn feathers; 3 = small bare patches (<30 mm); 4 = large bare patches (>30 mm); and 5 = no feathers in most body areas. Data are presented as median (Q1-Q3). From 16 to 28 weeks of age, all birds across housing systems had a feather score of 1 and were therefore excluded from statistical analysis due to lack of variation. Differences among housing systems at each age were evaluated using the Kruskal-Wallis test. No significant differences were detected at any age ($P > 0.05$).

Foot lesion scores of birds at different ages:

Foot lesion scores across housing systems are summarized in Table 2. At 16, 20, and 24 weeks of age, all birds recorded the minimum lesion score (score 1), indicating the absence of detectable foot lesions and preventing meaningful statistical comparison among treatments.

From 28 weeks onward, lesion scores increased slightly with age across all housing systems. Nevertheless, no significant differences were observed among individual cages, colony cages, and deep litter housing at 28 weeks, 32 weeks, 36 weeks, or 40 weeks of age. At 40 weeks, birds housed in individual cages showed numerically higher lesion scores (2.4 ± 0.37) compared with colony cages (1.9 ± 0.28) and deep litter housing (1.5 ± 0.22); however, the variation remained statistically non-significant.

These findings suggest that foot lesion severity increased moderately with age but was not significantly influenced by housing system during the experimental period.

Table 2: Foot Lesion Score, across Housing Systems at Different Ages

Age (weeks)	Individual Cage	Colony Cage	Deep Litter	H (df = 2)	P-value
28	1.0 (1.0–1.0)	1.0 (1.0–1.0)	1.0 (1.0–1.0)	2.00	0.368
32	1.0 (1.0–2.0)	1.0 (1.0–2.0)	1.0 (1.0–1.0)	0.52	0.771
36	1.5 (1.0–2.0)	1.0 (1.0–2.0)	1.0 (1.0–1.5)	2.41	0.300
40	2.0 (1.0–3.0)	1.5 (1.0–2.5)	1.0 (1.0–2.0)	3.61	0.164

Foot lesion scores ranged from 1 (no lesions) to 5 (severe lesions). Data are presented as median (Q1-Q3). Birds from all housing systems exhibited a score of 1 between 16 and 24 weeks of age; therefore, these ages were not subjected to statistical comparison due to absence of variability. Differences among housing systems at each age were assessed using the Kruskal-Wallis test. No significant differences were detected ($P > 0.05$).

Keel bone deformities:

Keel bone deformity scores are presented in Table 3. Minimal deformities were observed at 32 and 36 weeks of age, with mean scores remaining close to the normal condition across all housing systems. No significant differences were detected among treatments at 32 weeks or 36 weeks. At 40 weeks, deformity scores increased across all groups, with the highest numerical value recorded in birds housed in individual cages (2.1 ± 0.37), followed by colony cages (1.6 ± 0.28) and deep litter housing (1.2 ± 0.22). Despite this numerical pattern, differences among housing systems did not reach statistical significance. Overall, the results indicate that keel bone deformities tended to increase with advancing age, whereas the influence of housing system was not statistically evident within the experimental population evaluated.

Table 3. Keel bone deformity scores of birds under different housing systems at various ages

Age (weeks)	Individual Cage	Colony Cage	Deep Litter	H (df = 2)	P-value
32	1.0 (1.0–1.0)	1.0 (1.0–1.0)	1.0 (1.0–1.0)	2.00	0.446
36	1.0 (1.0–2.0)	1.0 (1.0–2.0)	1.0 (1.0–1.0)	1.22	0.370
40	2.0 (1.0–3.0)	1.5 (1.0–2.0)	1.0 (1.0–2.0)	4.46	0.115

Keel bone deformity scores were assessed on an ordinal scale, with higher scores indicating greater deformity severity. Data are presented as median (Q1-Q3). Differences among housing systems at each age were analyzed using the Kruskal-Wallis test. No significant differences were observed at any age ($P > 0.05$).

Gait Performance across Housing Systems

The distribution of gait scores across housing systems and ages is presented in Table 4. All birds exhibited normal locomotion (gait score 0) between 16 and 28 weeks of age, indicating the absence of observable walking impairment during the early production period. From 32 weeks onward, mild gait abnormalities began to appear in some birds. At 32 weeks, 20% of birds housed in individual cages and 10% of birds housed in colony cages exhibited a gait score of 1, whereas all birds maintained under the deep litter system retained normal gait. The prevalence of mild gait impairment increased further at 36 and 40 weeks across all housing systems. By 40 weeks of age, a greater proportion of birds housed in individual cages exhibited impaired gait scores compared with birds maintained in colony cages and deep litter housing. Deep litter birds generally showed a higher proportion of normal gait scores and fewer birds with moderate impairment. However, because no formal statistical comparison was performed for gait score distributions, these observations should be interpreted as descriptive trends rather than evidence of significant housing effects. No birds in any treatment group exhibited severe gait impairment (scores 4 or 5) during the study period.

Table 4. Gait performance across housing systems at different bird ages

Age (weeks)	Individual Cage	Colony Cage	Deep Litter	H (df = 2)	P-value
32	0.0 (0.0–0.0)	0.0 (0.0–0.0)	0.0 (0.0–0.0)	2.15	0.341
36	0.0 (0.0–1.0)	0.0 (0.0–1.0)	0.0 (0.0–0.0)	1.06	0.589
40	0.5 (0.0–2.0)	0.0 (0.0–1.0)	0.0 (0.0–1.0)	2.01	0.364

Gait scores ranged from 0 (normal gait) to 5 (severe locomotor impairment). Data are presented as median (Q1-Q3). All birds exhibited a gait score of 0 between 16 and 28 weeks of age; therefore, these ages were excluded from statistical analysis because no variation existed among observations. Differences among housing systems at each age were evaluated using the Kruskal-Wallis test. No significant differences were detected ($P > 0.05$).

Claw length:

Claw length differed significantly among housing systems ($P < 0.05$; Table 5). Birds maintained in the deep litter system exhibited the shortest claws (57.4 ± 0.6 mm), whereas birds housed in individual cages had the greatest claw length (61.8 ± 1.03 mm). Claw length in colony cages (60.1 ± 0.6 mm) was intermediate and did not differ significantly from either deep litter or individual cage systems.

Table 5: Claw length of birds under different housing systems

Housing System	Claw Length (mm)
Deep Litter	57.4 ^a ±0.6
Colony Cage	60.1 ^{ab} ±0.6
Individual Cage	61.8 ^b ±1.03

Means with different superscripts in a column differ significantly (P<0.05).

Tonic immobility:

The duration of tonic immobility differed significantly among housing systems (P < 0.05; Table 6). Birds reared under the deep litter system exhibited the shortest tonic immobility duration (64.6 ± 10.69 s), followed by colony cage birds (72.1 ± 10.18 s). The longest duration was recorded in birds housed in individual cages (171.3 ± 24.13 s). No significant difference was observed between the deep litter and colony cage groups.

Table 6: Average duration (s) of tonic immobility displayed by birds in different housing system

Housing System	Tonic Immobility (Sec)
Deep Litter	64.6 ^a ±10.69
Colony Cage	72.1 ^a ±10.18
Individual Cage	171.3 ^b ±24.13

Means with different superscripts in a column differ significantly (P<0.05).

Blood biochemical and hormonal profile:

The effects of housing system on biochemical, hematological, and hormonal variables at 16 and 40 weeks of age are presented in Table 7. Most measured parameters, including serum glucose, total protein, albumin, globulin, uric acid, aspartate aminotransferase (AST), alanine aminotransferase (ALT), total cholesterol, high-density lipoprotein (HDL), low-density lipoprotein (LDL), and thyroxine (T4), did not differ significantly among housing systems at either sampling age (P > 0.05). Serum glucose concentrations ranged from 229 to 240 mg/dL at 16 weeks and from 237 to 255 mg/dL at 40 weeks, while total protein concentrations remained within a relatively narrow range across treatments. Similarly, liver function indicators (AST, ALT, and uric acid), lipid profile components, and serum protein fractions showed no significant housing-related differences throughout the study. Among the hormonal variables, triiodothyronine (T3) concentrations differed significantly among housing systems at 40 weeks of age (P < 0.05). Birds maintained under the deep litter system recorded higher T3 concentrations (148 ng/dL) than birds housed in individual cages (127 ng/dL) and colony cages (124 ng/dL). However, no significant

differences in T3 were observed at 16 weeks, and T4 concentrations remained comparable among treatments at both ages.

Leukocyte differential counts were largely unaffected by housing system. Heterophil and lymphocyte percentages did not differ significantly among treatments at either age. However, the heterophil-to-lymphocyte (H/L) ratio was significantly influenced by housing system at both sampling periods ($P < 0.05$). At 16 weeks, birds housed in colony cages exhibited a lower H/L ratio (0.52) than birds maintained in individual cages (0.58) and deep litter housing (0.57). At 40 weeks, the lowest H/L ratio was observed in birds maintained under the deep litter system (0.52), whereas higher values were recorded in colony cages (0.61) and individual cages (0.60).

Serum corticosterone concentrations were similar among treatments at 16 weeks but differed significantly at 40 weeks of age ($P < 0.05$). Birds housed in the deep litter system exhibited the highest corticosterone concentration (2.54 ng/mL), followed by colony cages (2.19 ng/mL) and individual cages (1.94 ng/mL). Overall, housing system had limited effects on most biochemical variables measured in the present study. Significant differences were primarily observed for T3 concentration, H/L ratio, and corticosterone concentration at 40 weeks of age.

Table 7: Effect of housing systems on blood biochemical and hormonal profile in bird

Parameters	Time	Individual Cage	Colony Cage	Deep Litter	SEM
Glucose (mg/dL)	16 th Week	240	236	229	4.55
	40 th Week	237	242	255	7.29
Total Protein (g/dL)	16 th Week	3.58	3.49	3.36	0.13
	40 th Week	3.55	3.52	3.46	0.28
T-3 (ng/dL)	16 th Week	125	119	123	7.8
	40 th Week	127 ^a	124 ^a	148 ^b	6.3
T-4(ng/mL)	16 th Week	1.48	1.45	1.47	0.11
	40 th Week	1.52	1.46	1.51	0.19
Uric Acid (mg/dL)	16 th Week	4.74	4.62	4.73	0.76
	40 th Week	4.87	4.77	4.51	0.92
AST(U/L)	16 th Week	146	153	149	6.11
	40 th Week	140	156	144	5.82
ALT(U/L)	16 th Week	30	29	33	1.12
	40 th Week	28	32	29	1.03
LDL (mg/dL)	16 th Week	25	28	24	0.83
	40 th Week	22	23	27	0.54
HDL (mg/dL)	16 th Week	81	85	88	1.46
	40 th Week	78	81	82	2.01
Cholesterol (mg/dL)	16 th Week	82	78	81	2.06
	40 th Week	87	83	74	2.33
Albumin (g/dL)	16 th Week	2.00	2.14	2.04	0.07
	40 th Week	2.11	2.03	2.12	0.03
Globulin (g/dL)	16 wk	1.58	1.35	1.32	0.8
	40 wk	1.44	1.49	1.34	0.5

Heterophil%	16 th Week	31.79	27.81	26.7	1.99
	40 th Week	30.75	31.32	30.16	1.63
Lymphocyte%	16 th Week	48.41	52.72	44.85	2.22
	40 th Week	51.11	50.97	54.28	2.01
H/L ratio	16 th Week	0.58 ^a	0.52 ^b	0.57 ^a	0.05
	40 th Week	0.60 ^a	0.61 ^a	0.52 ^b	0.03
Corticosterone (ng/ml)	16 th Week	1.804	1.812	1.99	0.04
	40 th Week	1.94 ^a	2.187 ^{ab}	2.54 ^b	0.05

Means with different superscripts in a column differ significantly ($P < 0.05$). a,b significant across the different housing system. T-3-Triiodothyronine; T-4-thyroxine, AST-aspartate aminotransferase; ALT-alanine aminotransferase; LDL-low density lipoprotein; HDL-high density lipoprotein.

Discussion:

Feather condition

Feather condition is widely regarded as an important indicator of poultry welfare, reflecting the combined effects of health status, behavioural interactions, environmental quality, and management practices (Gunnarsson, 1999); (Lambton et al., 2013). In the present study, feather scores did not differ significantly among housing systems, although hens reared in the deep litter system showed numerically better plumage condition at later ages. A gradual decline in feather quality was observed across all treatments with advancing age, suggesting that age-related feather wear may have contributed more to plumage deterioration than housing environment. Similar observations have been reported in previous studies where feather quality declined progressively during the laying cycle irrespective of housing type (Tauson et al., 1999; Tauson, 2005) which highlighted the welfare advantages of more enriched housing environments. Conversely, feather damage has often been associated with housing environments that restrict behavioural activities or increase contact with cage components (Tactacan et al., 2009) and (Bist et al., 2023), indicating that restricted housing systems may suggest that confinement and environmental restrictions inherent to these systems may contribute to feather wear and damage. Similarly, (Pichová et al., 2017) observed greater feather deterioration in cage systems than in deep litter housing, while (Vasdal et al., 2023) reported increased plumage damage under certain intensive housing conditions. However, the absence of significant differences in the present study suggests that appropriate nutrition, environmental enrichment, and management practices may mitigate housing-related effects on feather condition. In contrast, free-range systems afford birds greater mobility, which facilitates physical exercise, enhances blood circulation and metabolic activity, and may

thereby promote feather growth and regeneration (Hartcher and Jones, 2017). Collectively, these findings underscore the integral role of housing systems in shaping feather condition and, by extension, the overall welfare status of laying hens.

Foot lesion scores

The majority of hens across all housing systems recorded a foot condition score of 1, indicative of excellent foot health and reflecting the effectiveness of management practices in place. Nevertheless, a decline in foot health over time was observed among birds housed in Individual and Colony Cages, pointing to possible welfare compromises associated with these systems. In contrast, hens maintained under the Deep Litter system demonstrated consistently optimal foot conditions, corroborating findings by (Abrahamsson and Tauson, 1995) and (Weitzenb rger et al., 2006), though differing from earlier observations reported by (Wang et al., 1998). The frequency of foot lesions was markedly lower in Deep Litter systems, suggesting that substrate type and environmental enrichment play critical roles in foot health. Soft, natural bedding materials in deep litter environments likely mitigate mechanical stress on the plantar surface of the feet, thereby reducing the incidence of pressure-related lesions. These results reinforce the significance of appropriate flooring materials and housing conditions in minimizing foot injuries and enhancing animal welfare. Moreover, (Honig et al., 2024) and (Topal and Petek, 2021) noted that despite the thermal efficiency and growth advantages associated with caged systems, the restriction of natural movement often led to a higher prevalence of leg injuries. These observations collectively advocate for the prioritization of mobility and natural behaviors in housing design to promote skeletal integrity and welfare in laying hens.

Keel bone deformities

Keel bone integrity varied considerably across housing systems. While the Colony Cage system yielded better outcomes than the Individual Cage system, a progressive decline in keel bone health was still observed. Although offering slightly more space, the Colony Cage did not suffice to prevent structural deformities over time. In contrast, the Deep Litter system consistently resulted in the most favorable keel bone health profiles, with minimal deviations recorded. These outcomes are consistent with findings by (Eusemann et al., 2018) (Rufener and Makagon, 2020); (DePaoli et al., 2024) who reported higher incidences of keel bone fractures in birds housed within caged systems compared to those reared in free-range environments. Enhanced physical activity and load-bearing movement associated with more open systems are believed to improve bone strength and structural resilience. However, (Pullin et al., 2024) found no substantial long-term effect of pullet rearing systems on subsequent keel bone fracture rates or on the use of aviary structures, apart from minor early variations in perch utilization. These nuances highlight the importance of evaluating both rearing and adult housing systems in a comprehensive manner (Toscano et al., 2020). When comparing Individual, Colony, and Deep Litter systems, it is apparent that the latter facilitates the expression of a wide range of natural behaviors-such as walking, scratching, dust bathing, and wing flapping-through the provision of unobstructed floor space. Such behaviors are vital for maintaining musculoskeletal health and minimizing trauma-related

keel bone injuries. Furthermore, deep litter systems inherently reduce the risk of collision injuries, as vertical transitions are minimal and stress-induced behaviors are less prevalent. Thus, the data strongly support the conclusion that enriched, non-cage systems are more conducive to skeletal health and overall welfare in laying hens. Moreover (Saraiva et al., 2019) reported increased prevalence of keel bone fracture in laying birds in caged system as compared to free range and barn system of rearing strengthening the claims of present finding.

Gait performance test:

These observations align with (Knowles et al., 2008), who identified gait abnormalities as a prominent welfare issue across various poultry housing systems, with variation in severity and frequency. Consistent with previous studies (Kestin et al., 2001), and (Knowles et al., 2008) restricted space and limited opportunities for physical activity were associated with compromised leg health and impaired mobility. Among the housing systems evaluated, birds in the Deep Litter system exhibited the best gait performance, followed by those in Colony Cages, while birds in Individual Cages showed the most significant decline, particularly in the later stages of the study. These results are aligned with the observations of (Vits et al., 2005), who suggested that excessive perch use in enriched cages may contribute to leg deformities. On contrary, (Ibrahim et al., 2023) reported no significant differences in gait performance among turkeys reared in different housing systems. Nevertheless, the present study underscores the importance of an enriched environment-such as that provided by the Deep Litter system-in promoting natural movement, reducing stress, and maintaining leg health. In contrast, the confinement and lack of stimulation in Individual Cages likely contributed to rapid and more severe gait deterioration, reinforcing the critical influence of housing design on poultry welfare.

Claw length:

The regulation of claw length is a key indicator of animal welfare, as excessively long claws can result in discomfort, injury, and impaired mobility. The results of this study aligns with those of (Vits et al., 2005) highlighting the importance of housing environments that encourage natural behaviors to mitigate claw overgrowth. Birds in the Deep Litter and Colony Cage systems, both of which offer more enriched settings, exhibited shorter claw lengths, indicative of better welfare conditions compared to those in Individual Cages. Housing characteristics such as space availability, flooring type, and access to perches play a pivotal role in facilitating natural behaviors like scratching and foraging, which help regulate claw length. The longer claws observed in birds from Individual Cages likely result from behavioral restriction and a lack of environmental stimuli, raising concerns about the potential for claw entanglement, injury, and subsequent mobility issues. These findings stress the importance of regular claw monitoring and the inclusion of enrichment strategies-such as perch provision and textured flooring—to maintain optimal claw health, particularly in restrictive housing systems.

Tonic immobility:

Tonic immobility (TI) is a widely used behavioural indicator of fearfulness and stress in poultry, with longer durations generally reflecting a greater fear response. In the present study, birds housed in individual cages exhibited significantly longer TI durations than those maintained in colony cages and deep litter systems, indicating a higher level of fear-related behaviours under more restrictive housing conditions. Birds reared in the deep litter system showed the shortest TI duration, while colony cages produced intermediate responses. These findings are consistent with earlier reports linking enriched environments and greater behavioural opportunities with reduced fear responses in laying hens (XI European Symposium on Poultry Welfare (ESPW 2023) et al., 2023),(Hartcher et al., 2016; Hartcher and Jones, 2017) . However, some inconsistencies remain in the literature. (Baxter et al., 2019) and (Adler et al., 2020) found that enrichment strategies like perch provision could effectively reduce fear responses, regardless of range access. Recent research by (Fiorilla et al., 2024) further observed no significant difference in TI duration between free-range and cage systems, though TI duration increased with age in caged birds, suggesting cumulative stress effects over time. Collectively, these findings underscore the importance of housing systems that facilitate natural behaviors and minimize stress-induced fear responses.

Blood biochemical profile and cortisone level

Most serum biochemical parameters, including glucose, total protein, albumin, globulin, AST, ALT, LDL, HDL, and total cholesterol, were not significantly affected by housing system. These findings indicate that the different housing environments evaluated in this study did not substantially alter general metabolic status or liver function. Our findings on blood biochemical parameters are consistent with previous studies. (Abdel-Latif et al., 2020) observed that chickens reared in either cage or floor systems showed no significant differences in blood characteristics such as total protein, globulin, albumin, AST, ALT, and total glucose levels. Similarly, (Abo Ghanima et al., 2020) reported that broiler chickens raised in cages, litter, or plastic slate systems exhibited no significant variations in AST and ALT. Furthermore, (Eid et al., 2025) found that broilers reared in cage or floor systems did not show significant differences in serum total protein, globulin, albumin, and glucose levels.

Lipoproteins and Cholesterol:

Low-density lipoprotein (LDL) and high-density lipoprotein (HDL) levels were stable across housing systems at both time points, with only slight, non-significant variations. Cholesterol levels showed a minor decline in birds housed on Deep Litter by the 40th week (74 mg/dL) compared to the cage systems. This reduction could suggest improved lipid metabolism in birds with greater freedom of movement, although further investigation is needed to confirm this observation. Conversely, (Elsayed et al., 2024) observed lower cholesterol and lipid profile in the birds owing to more movements in the free range system of poultry rearing as compared to caged birds. Further, (Özbey and Esen, 2007) observed a notable decrease in serum total cholesterol levels in rock partridges reared on the ground compared to those housed in cages.

Thyroid Hormones (T3 and T4):

Housing system influenced serum T3 concentration, whereas T4 remained unaffected. Birds maintained under the deep litter system exhibited significantly higher T3 levels at 40 weeks compared with those housed in individual and colony cages. As T3 is the biologically active thyroid hormone involved in regulating metabolic activity, this response may reflect differences in physical activity, environmental interaction, and behavioural expression among housing systems. The greater opportunity for movement and exploration in the deep litter environment may have contributed to the elevated T3 concentrations observed in these birds. Previous studies have reported variable effects of housing and environmental stress on thyroid hormone dynamics. For example (Abdel-Hamid and Dawod, 2020) observed higher free T4 concentrations in ducks reared under cage systems than in litter-based systems, suggesting that housing-related stress can influence thyroid function. In contrast (Abo El-Maaty et al., 2023) reported reductions in both T3 and T4 concentrations in birds exposed to stress. The inconsistency among studies indicates that thyroid responses are influenced by multiple factors, including species, age, management conditions, and the nature of the stressor. Therefore, while the higher T3 concentration observed in deep litter birds may indicate altered metabolic activity, its biological significance should be interpreted cautiously.

Heterophil/Lymphocyte Ratio:

The heterophil-to-lymphocyte (H/L) ratio is widely used as an indicator of chronic stress and physiological adaptation in poultry. In the present study, birds housed in the deep litter system exhibited the lowest H/L ratio at 40 weeks of age, whereas slightly higher values were observed in colony and individual cage systems. Although the magnitude of the difference was relatively small, the findings suggest a more favorable stress response in birds maintained under the deep litter environment during the later stage of production. These observations are consistent with earlier reports showing elevated H/L ratios in housing systems that restrict behavioural expression (Wall et al., 2004). In the same line (Erek and Matur, 2025) found that hens in furnished cages and deep-litter systems exhibited significantly lower H/L ratios than those in conventional cages and free-range systems. These findings align with (Barnett et al., 2009) and (Tainika et al., 2024) observed greater stress markers in caged housing system as compared to floor-reared birds. These observations are consistent with earlier reports showing elevated H/L ratios in housing systems that restrict behavioural expression.

Corticosterone Levels:

Corticosterone is a key endocrine marker associated with activation of the hypothalamic-pituitary-adrenal (HPA) axis and is commonly used to assess physiological responses to environmental challenges. In the present study, birds housed in the deep litter system exhibited significantly higher corticosterone concentrations at 40 weeks than birds maintained in individual cages, while colony cage birds showed intermediate values. Previous studies have reported contrasting effects of housing systems on corticosterone responses. For instance (Erek and Matur, 2025) reported that hens in cage systems experienced fewer stressors than those in floor pens, possibly due to the more controlled environment of cages. Elevated corticosterone concentrations have also been associated with adverse

environmental conditions, social interactions, and increased stocking density. (Bessei, 2018),(Pavlik et al., 2008)(Kang et al., 2016)(Honig et al., 2024).In contrast, (Campbell et al., 2022) reported higher corticosterone levels in caged layers than in pen-housed birds, highlighting the complexity of endocrine responses across production systems.

An important observation in the present study is that the higher corticosterone concentration in deep litter birds occurred alongside shorter tonic immobility durations and lower H/L ratios. This suggests that individual welfare indicators may not always respond in the same direction and may reflect different aspects of adaptation to the housing environment. Consequently, corticosterone should be interpreted together with behavioural and hematological measures rather than as a standalone indicator of welfare status. The findings highlight the multifaceted nature of stress responses in laying hens and reinforce the need for a holistic approach when assessing poultry welfare under different housing systems.

CONCLUSIONS

The present study evaluated the effects of individual cage, colony cage, and deep litter housing systems on behavioural, physical, hematological, biochemical, and hormonal indicators of welfare in Rhode Island Red hens. Housing system influenced several welfare-related parameters, particularly tonic immobility duration, claw length, serum T3 concentration, and H/L ratio. Birds reared under the deep litter system exhibited shorter tonic immobility durations, reduced claw length, higher T3 concentrations, and lower H/L ratios at 40 weeks of age, suggesting improved behavioural adaptation and a more favourable physiological status. In contrast, most biochemical parameters, feather condition scores, foot lesion scores, and keel bone deformity scores were not significantly affected by housing system under the management conditions of the present study. An important finding was that welfare indicators did not always respond uniformly across housing systems. Although birds maintained in the deep litter system showed favourable behavioural and hematological responses, they also exhibited higher corticosterone concentrations at the later stage of production. This highlights the complexity of welfare assessment and emphasizes the need to interpret individual indicators within a broader physiological and behavioural context. Overall, the findings suggest that housing systems that allow greater behavioural expression may offer welfare advantages for Rhode Island Red hens under tropical production conditions. However, the observed responses indicate that no single parameter can fully characterize welfare status. Future studies incorporating larger populations, longer production cycles, and additional welfare biomarkers are needed to better understand the interactions among housing environment, physiological adaptation, and bird welfare.

Declarations

ACKNOWLEDGEMENT

The authors would like to express their gratitude to the Poultry Research & Training Centre, BASU, Patna, for providing the facilities and support necessary to carry out this research. Special thanks to the staff

and faculty for their assistance and expertise throughout this study.

Author Contribution

The study was conceived and designed by A. Kumar, R. R. K. Sinha, and K Kumar. The methodology was developed by R. R. K. Sinha and S. P. Sahu. Investigation and data collection were carried out by A. Kumar, A K Bharti and K. Kumar. Formal analysis and data curation were performed by A. Kumar, A K Bharti and A. K. Singh. Resources were provided by R. R. K. Sinha and S. p. Sahu. The original draft of the manuscript was written by A. Kumar and A. K. Singh. Review and editing of the manuscript were conducted by R. R. K. Sinha, S. P. Sahu, K. Kumar, and A. Bharti. Visualization was done by A. K. Singh. All authors have read and approved the final manuscript and agree to be accountable for all aspects of the work.

Funding

This research received no external funding.

Data Availability Statement

The datasets generated and analyzed during the current study are not publicly available but are available from the corresponding author upon reasonable request. All relevant data supporting the findings of this study are included within the manuscript.

Ethical Approval

All experimental procedures involving animals were conducted in accordance with the guidelines for the care and use of animals in research and were approved by the Institutional Animal Ethics Committee (IAEC) of Bihar Animal Sciences University (BASU), Patna, Bihar, India (Approval No.: IAEC/BVC/2024/31).

CONFLICT OF INTEREST

We certify that there is no conflict of interest with any financial, personal, or other relationships with other people or organization related to the material discussed in the manuscript.

References

1. Abdel-Hamid, T.M. and Dawod, A., 2020. Breed Effects on Growth Performance, Blood Parameters and the Levels of Metabolic Hormones in Rabbits Under Heat Stress in Egypt Zagazig Veterinary Journal, 48, 284–295
2. Abdel-Latif, E.A., Ibrahim, Z.A., Reda, F.M. and Alagawany, M., 2020. Effect of *Aspergillus japonicas* culture filtrate on performance, carcass yield, digestive enzymes, intestinal microbiota and blood constituents of quail Italian Journal of Animal Science, 19, 1057–1064

3. Abo El-Maaty, H., Sherif, S., Taha, A.E., Al-Otaibi, A.M., Othman, S.I., Allam, A.A. and Mahrose, K., 2023. Effects of housing systems and feed additive on growth, carcass traits, liver function, oxidative status, thyroid function, and immune parameters of broilers *Poultry Science*, 102, 103121
4. Abo Ghanima, M.M., Elsadek, M.F., Taha, A.E., Abd El-Hack, M.E., Alagawany, M., Ahmed, B.M., Elshafie, M.M. and El-Sabrout, K., 2020. Effect of Housing System and Rosemary and Cinnamon Essential Oils on Layers Performance, Egg Quality, Haematological Traits, Blood Chemistry, Immunity, and Antioxidant Animals, 10, 245
5. Abrahamsson, P. and Tauson, R., 1995. Aviary Systems and Conventional Cages for Laying Hens: Effects on Production, Egg Quality, Health and Bird Location in Three Hybrids *Acta Agriculturae Scandinavica, Section A – Animal Science*, 45, 191–203
6. Abrahamsson, P., Tauson, R. and Appleby, M.C., 1996. Behaviour, health and integument of four hybrids of laying hens in modified and conventional cages *British Poultry Science*, 37, 521–540
7. Adler, C., Tiemann, I., Hillemacher, S., Schmithausen, A.J., Müller, U., Heitmann, S., Spindler, B., Kemper, N. and Büscher, W., 2020. Effects of a partially perforated flooring system on animal-based welfare indicators in broiler housing *Poultry Science*, 99, 3343–3354
8. Barnett, J.L., Tauson, R., Downing, J.A., Janardhana, V., Lowenthal, J.W., Butler, K.L. and Cronin, G.M., 2009. The effects of a perch, dust bath, and nest box, either alone or in combination as used in furnished cages, on the welfare of laying hens *Poultry Science*, 88, 456–470
9. Baxter, M., Bailie, C.L. and O’Connell, N.E., 2019. Play behaviour, fear responses and activity levels in commercial broiler chickens provided with preferred environmental enrichments *Animal*, 13, 171–179
10. Baylan, M., Kursun, K., Abdallah, N., Celik, L., Yenilmez, F. and Kutay, H., 2024. The Effect of Housing Systems on the Growth, Egg production, Overall Egg Weight and Egg Quality Traits of a New Turkish Laying Hen Hybrid, *Akbay Brazilian Journal of Poultry Science*, 26, eRBCA-2024-1924
11. Bessei, W., 2018. Impact of animal welfare on worldwide poultry production *World’s Poultry Science Journal*, 74, 211–224
12. Bhadauria, P., Bhanja, S.K., Kolluri, G., Saran, S., Divya and Majumdar, S., 2019. Productivity, reproduction and welfare status of White Leghorn hens under cage and floor housing systems *Indian Journal of Poultry Science*, 54, 89
13. Bist, R.B., Subedi, S., Yang, X. and Chai, L., 2023. Effective Strategies for Mitigating Feather Pecking and Cannibalism in Cage-Free W-36 Pullets *Poultry*, 2, 281–291
14. Blatchford, R.A., Fulton, R.M. and Mench, J.A., 2016. The utilization of the Welfare Quality® assessment for determining laying hen condition across three housing systems *Poultry Science*, 95, 154–163
15. Campbell, A.M., Johnson, A.M., Persia, M.E. and Jacobs, L., 2022. Effects of Housing System on Anxiety, Chronic Stress, Fear, and Immune Function in Bovan Brown Laying Hens *Animals*, 12, 1803
16. Campo, J.L., Prieto, M.T. and Dávila, S.G., 2008. Effects of Housing System and Cold Stress on Heterophil-to-Lymphocyte Ratio, Fluctuating Asymmetry, and Tonic Immobility Duration of Chickens

17. DePaoli, E., Korver, D. and Bench, C., 2024. Effect of pullet rearing environment, strain and perch shape on perching behaviour, perching biomechanics, and keel bone damage in enriched-housed laying hens *Applied Animal Behaviour Science*, 272, 106187
18. Eid, K., Gabr, S. and Gouda, A., 2025. Immunity, Haematology and Biochemical analysis of Ross 308 and Avian 48 Broiler Strains under Different Egyptian Environmental Conditions and Housing Systems *Journal of Productivity and Development*, 30, 267–281
19. Elsayed, R.A., Mohammed, A.S., Abou-Elnaga, A.F., Abou-Ismael, U.A., Darwish, R.A. and Fouda, M.M., 2024. EFFECTS OF DIFFERENT TYPES OF ENVIRONMENTAL ENRICHMENT ON BEHAVIOUR, PERFORMANCE, WELFARE, CARCASS CHARACTERISTICS AND LIPID PROFILE OF BROILER CHICKENS *Mansoura Veterinary Medical Journal*, 25
20. Ereke, M. and Matur, E., 2025. Effects of different housing systems on the oxidative defence system, heterophil functions, cellular immune response and cytokines in laying hens *British Poultry Science*, 66, 1–9
21. Ereke, M. and Matur, E., 2024. Effects of Housing Systems on Production Performance, Egg Quality, Tonic Immobility and Feather Score in Laying Hens *Veterinary Medicine and Science*, 10, e70112
22. Eusemann, B.K., Baulain, U., Schrader, L., Thöne-Reineke, C., Patt, A. and Petow, S., 2018. Radiographic examination of keel bone damage in living laying hens of different strains kept in two housing systems *PLOS ONE*, 13, e0194974
23. Fiorilla, E., Ozella, L., Sirri, F., Zampiga, M., Piscitelli, R., Tarantola, M., Ponzio, P. and Mugnai, C., 2024. Effects of housing systems on behaviour and welfare of autochthonous laying hens and a commercial hybrid *Applied Animal Behaviour Science*, 274, 106247
24. Gunnarsson, S., 1999. Effect of rearing factors on the prevalence of floor eggs, cloacal cannibalism and feather pecking in commercial flocks of loose housed laying hens *British Poultry Science*, 40, 12–18
25. Hartcher, K.M., Hickey, K.A., Hemsworth, P.H., Cronin, G.M., Wilkinson, S.J. and Singh, M., 2016. Relationships between range access as monitored by radio frequency identification technology, fearfulness, and plumage damage in free-range laying hens *Animal*, 10, 847–853
26. Hartcher, K.M. and Jones, B., 2017. The welfare of layer hens in cage and cage-free housing systems *World's Poultry Science Journal*, 73, 767–782
27. Honig, H., Haron, A., Plitman, L., Lokshtanov, D., Shinder, D., Nagar, S., Goshen, T. and Druyan, S., 2024. Comparative Analysis of Broiler Housing Systems: Implications for Production and Wellbeing *Animals*, 14, 1665
28. Hüttner, J., Clauß, A., Klambeck, L., Andersson, R., Kemper, N. and Spindler, B., 2023. Association with Different Housing and Welfare Parameters on Results of a Novel Object Test in Laying Hen Flocks on Farm Animals, 13, 2207
29. Ibrahim, R.R., Abdel Azeem, N.M., Emeash, H. and Abdelghany, A.K., 2023. Performance, behavior, and welfare of turkey poults reared under different housing conditions *Journal of Advanced*

30. Jones, R.B. and Faure, J.M., 1981. Sex and strain comparisons of tonic immobility (“Righting time”) in the domestic fowl and the effects of various methods of induction Behavioural Processes, 6, 47–55
31. Kang, H.K., Park, S.B., Kim, S.H. and Kim, C.H., 2016. Effects of stock density on the laying performance, blood parameter, corticosterone, litter quality, gas emission and bone mineral density of laying hens in floor pens Poultry Science, 95, 2764–2770
32. Kestin, S., Knowles, T., Tinch, A. and Gregory, N., 1992. Prevalence of leg weakness in broiler chickens and its relationship with genotype Veterinary Record, 131, 190–194
33. Kestin, S.C., Gordon, S., Su, G. and Sørensen, P., 2001. Relationships in broiler chickens between lameness, liveweight, growth rate and age Veterinary Record, 148, 195–197
34. Knowles, T.G., Kestin, S.C., Haslam, S.M., Brown, S.N., Green, L.E., Butterworth, A., Pope, S.J., Pfeiffer, D. and Nicol, C.J., 2008. Leg Disorders in Broiler Chickens: Prevalence, Risk Factors and Prevention PLoS ONE, 3, e1545
35. Kraus, A., Krunt, O., Zita, L., Vejvodová, K. and Drábek, O., 2022. Laying hens under smallholder conditions: laying performance, growth and bone quality of tibia and femur including essential elements Poultry Science, 101, 101927
36. Kraus, A., Zita, L., Krunt, O., Härtlová, H. and Chmelíková, E., 2021. Determination of selected biochemical parameters in blood serum and egg quality of Czech and Slovak native hens depending on the housing system and hen age Poultry Science, 100, 1142–1153
37. Lambton, S.L., Nicol, C.J., Friel, M., Main, D.C.J., McKinsty, J.L., Sherwin, C.M., Walton, J. and Weeks, C.A., 2013. A bespoke management package can reduce levels of injurious pecking in loose-housed laying hen flocks Veterinary Record, 172, 423–423
38. Mashaly, M.M., Hendricks, G.L., Kalama, M.A., Gehad, A.E., Abbas, A.O. and Patterson, P.H., 2004. Effect of Heat Stress on Production Parameters and Immune Responses of Commercial Laying Hens Poultry Science, 83, 889–894
39. Nicol, C.J., Brown, S.N., Glen, E., Pope, S.J., Short, F.J., Warriss, P.D., Zimmerman, P.H. and Wilkins, L.J., 2006. Effects of stocking density, flock size and management on the welfare of laying hens in single-tier aviaries British Poultry Science, 47, 135–146
40. Özbey, O. and Esen, F., 2007. The Effects of Breeding Systems and Stocking Density on Some Blood Parameters of Rock Partridges (*Alectoris graeca*) Poultry Science, 86, 420–422
41. Pavlik, A., Jezova, D., Zapletal, D., Bakos, J. and Jelinek, P., 2008. Impact of housing technology on blood plasma corticosterone levels in laying hens Acta Veterinaria Hungarica, 56, 515–527
42. Pichová, K., Bilčík, B. and Košťál, L., 2017. Assessment of the effect of housing on feather damage in laying hens using IR thermography Animal, 11, 661–669
43. Pires, P.G.D.S., Bavaresco, C., Prato, B.S., Wirth, M.L. and Moraes, P.D.O., 2021. The relationship between egg quality and hen housing systems - A systematic review Livestock Science, 250, 104597

44. Pullin, A.N., Rufener, C.B., Millman, S.T., Tarlton, J.F., Toscano, M.J., Blatchford, R.A. and Makagon, M.M., 2024. Providing elevated structures in the pullet rearing environment affects behavior during initial acclimation to a layer aviary *Poultry Science*, 103, 103357
45. Rentsch, A.K., Ellis, J.L. and Widowski, T.M., 2023. Fearfulness in commercial laying hens: a meta-analysis comparing brown and white egg layers *Poultry Science*, 102, 102664
46. Rufener, C. and Makagon, M.M., 2020. Keel bone fractures in laying hens: a systematic review of prevalence across age, housing systems, and strains *Journal of Animal Science*, 98, S36–S51
47. Sabo, M.N., Usman, H.B., Mbakwe, C.C., Jibia, Z.S. and Sulaiman, A., 2025. Effect of Different Housing Systems and Sex on the Haematological and Serum Biochemical Indices of Locally Adapted and Exotic Turkeys Sahel *Journal of Life Sciences FUDMA*, 3, 23–29
48. Saraiva, S., Esteves, A. and Stilwell, G., 2019. Influence of different housing systems on prevalence of keel bone lesions in laying hens *Avian Pathology*, 48, 454–459
49. Sherwin, C.M., Richards, G.J. and Nicol, C.J., 2010. Comparison of the welfare of layer hens in 4 housing systems in the UK *British Poultry Science*, 51, 488–499
50. Sonkamble, V.V., 2020. Effect of Cage or Deep Litter Housing on Production Performance of White Leghorn Chickens *Journal of Animal Research*, 10
51. Susilo, A.Y.T.B., Hanif, M.F., Ariyadi, B., Putra, A.R.S. and Agus, A., 2024. Research Trends on Cage-Free Housing System in Laying Hens: A Bibliometric Analysis *Advances in Animal and Veterinary Sciences*, 12
52. Tactacan, G.B., Guenter, W., Lewis, N.J., Rodriguez-Lecompte, J.C. and House, J.D., 2009. Performance and welfare of laying hens in conventional and enriched cages *Poultry Science*, 88, 698–707
53. Tainika, B., Şekeroğlu, A., Akyol, A., Abaci, S.H. and Gür, F.M., 2024. The effect of housing environment (deep litter with or without access to different plant species outdoor) on welfare and behavior across two strains of laying hens *Turkish Journal of Veterinary & Animal Sciences*, 48, 1–16
54. Tauson, R., 2005. Management and housing systems for layers – effects on welfare and production *World's Poultry Science Journal*, 61, 477–490
55. Tauson, R., Wahlström, A. and Abrahamsson, P., 1999. Effect of Two Floor Housing Systems and Cages on Health, Production, and Fear Response in Layers *Journal of Applied Poultry Research*, 8, 152–159
56. Topal, E. and Petek, M., 2021. Effects of fully or partially slatted flooring designs on the performance, welfare and carcass characteristics of broiler chickens *British Poultry Science*, 62, 804–809
57. Toscano, M.J., Dunn, I.C., Christensen, J.-P., Petow, S., Kittelsen, K. and Ulrich, R., 2020. Explanations for keel bone fractures in laying hens: are there explanations in addition to elevated egg production? *Poultry Science*, 99, 4183–4194

58. Umber Rauf, Fatima Batool, Muhammad Usman, Muhammad Mohsan, Muhammad Ali Assad, Malik Mohsin Ali, Muhammad Mujtaba, Ghulam Murtaza Awan, Muhammad Ahsan Iftikhar, and Saqib Saleem Abdullah, 2024. Effect of Various Types of Housing Systems in Poultry Especially Broilers Indus Journal of Bioscience Research, 2, 600–607
59. Vasdal, G., Newberry, R.C., Estevez, I., Kittelsen, K. and Marchewka, J., 2023. The Aviary Transect—a practical welfare assessment tool to improve the management of cage-free laying hens Poultry Science, 102, 102659
60. Vits, A., Weitzenb rger, D., Hamann, H. and Distl, O., 2005. Production, egg quality, bone strength, claw length, and keel bone deformities of laying hens housed in furnished cages with different group sizes Poultry Science, 84, 1511–1519
61. Wall, H., Tauson, R. and Elwinger, K., 2004. Pop hole passages and welfare in furnished cages for laying hens British Poultry Science, 45, 20–27
62. Wang, G., Ekstrand, C. and Svedberg, J., 1998. Wet litter and perches as risk factors for the development of foot pad dermatitis in floor-housed hens British Poultry Science, 39, 191–197
63. Weitzenb rger, D., Vits, A., Hamann, H., Hewicker-Trautwein, M. and Distl, O., 2006. Macroscopic and histopathological alterations of foot pads of laying hens kept in small group housing systems and furnished cages British Poultry Science, 47, 533–543
64. Wilkins, L.J., McKinstry, J.L., Avery, N.C., Knowles, T.G., Brown, S.N., Tarlton, J. and Nicol, C.J., 2011. Influence of housing system and design on bone strength and keel bone fractures in laying hens Veterinary Record, 169, 414–414
65. XI European Symposium on Poultry Welfare (ESPW 2023), Trefil, P., Lichovn kov , M., and World's Poultry Science Association (eds), 2023. XI European Symposium on Poultry Welfare (ESPW 2023): proceedings: Prague, Czech Republic: 26-29 June 2023, (GUARANT: Prague)
66. Yilmaz Dikmen, B., İpek, A., Şahan,  ., Petek, M. and S zc , A., 2016. Egg production and welfare of laying hens kept in different housing systems (conventional, enriched cage, and free range) Poultry Science, 95, 1564–1572