



# Spatial distribution, movement, body damage, and feather condition of laying hens in a multi-tier system

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**ABSTRACT** The welfare and health of laying hens in the multitier system raise concern in public. The flock distributions during feeding time at 51 and 89 wk were studied in a multitier system. Furthermore, the ultra-high frequency radio frequency identification (**UHF RFID**) equipment was used to identify the transition between tiers and time spent in each tier of 48 focal hens (12 hens from each tier-group of the multitier system) at 92 wk of age. The body weight, tibia size (length and width), body damage (comb and rear part), and feather condition (neck, breast, back, tail, cloaca, and wings) of focal hens from different tier-groups were further compared. The results showed that the spatial distribution in flocks changed from top to bottom with increasing age. The hens at 51 wk of age were mainly distributed in the 4th tier ( $19.6 \pm 5.0\%$  in 1st tier,  $9.6 \pm 1.1\%$  in 2nd tier,  $23.6 \pm 2.9\%$  in 3rd tier and  $47.3 \pm 2.6\%$  in 4th tier), and hens at 89 wk of age were mainly distributed in the lower tiers ( $33.5 \pm 1.5\%$  in 1st tier,  $31.9 \pm 5.1\%$  in 2nd tier,  $15.7 \pm 3.4\%$  in 3rd tier and  $16.6 \pm 3.1\%$  in 4th tier). The spatial

distribution of hens at 89 wk of age was more even than that at 51 wk of age. At 92 wk of age, the proportion of time spent in original tier of 4 tier-groups was  $91.0 \pm 5.7\%$ ,  $51.9 \pm 5.7\%$ ,  $59.0 \pm 7.0\%$  and  $63.0 \pm 6.7\%$ , respectively. Focal hens preferred to stay in the original tier and spent significantly less time in other tiers ( $P < 0.05$ ). There was no significant difference in body weight, body damage score, tibia width and partial feather scores (neck, breast, tail, and cloaca) of focal hens among 4 tier-groups ( $P > 0.05$ ). However, focal hens from 1st tier had worse feather scores on wings and back, and shorter tibia length compared to other tiers suggesting that there were more lower ranking birds that located in lower tier to avoid competition, but had equal access to resource, which is good for their welfare and health. In summary, the overcrowding situation was improved near the end of the laying cycle in the multitier system, thereby mitigating the potential negative effects to the lower ranking hens and maintain a satisfactory level of welfare and health for laying hens near the end of the laying cycle.

**Key words:** multitier system, spatial distribution, UHF RFID, body damage, feather condition

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## INTRODUCTION

With the development of welfare-based farming models, livestock health and welfare levels are receiving more attention. The multitier system, as one of the alternatives to the traditional cage system, provides hens with multiple tiers of activity space (Stratmann et al., 2015), and fully satisfies the space usage for free movement and

behavioral expression (Hartcher and Jones, 2017). However, influencing factors such as the spatial design of breeding facilities (Arnould and Faure, 2004), resource allocation (Lentfer et al., 2013), and rank order of hens (Odén et al., 2004) may lead to the uneven spatial distribution of hens in a multitier system (Channing et al., 2001; Purdum et al., 2020). Moreover, one major trait currently being developed further is persistency of lay and the concept of the long life layer (Bain et al., 2016). The industry aims to extend egg laying until hens are 100-wk old or longer (from 65 to 70 wk currently) to make egg production more sustainable (Alfonso-Carrillo et al., 2021). As feather damage intensified (Lieber et al., 2019) and bone quality decreased (Hester et al., 2011) with increasing age, the locomotor ability of hens

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was inevitably affected, leading to changes in spatial activity patterns that may further exacerbate the uneven spatial distribution. Uneven spatial distribution of hens led to an increase in local group size, which could result in obstructed-free movement (Appleby et al., 1989) and increased individual fighting (Allen and Perry, 1975; Nicol et al., 1999; Zimmerman et al., 2006). Choudary et al. (1972) reported that agonistic acts (fighting, peck avoidance, threat avoidance, and avoidance) were associated with extensive feather loss. Feather loss was considered as a criterion of hen welfare and indicated the presence of stress (Campo et al., 2001). Therefore, it is crucial to clarify the spatial distribution and space usage of hens in the multitier systems to improve the health, welfare and production performance of hens.

At present, extensive research has been carried out on the spatial distribution and space usage of hens in different noncage systems to evaluate the welfare of birds. However, most of the studies did not track individual hens over time but instead did observations at the flock level or only at earlier age points. Carmichael et al. (2010) investigated the space usage of laying hens of 20 to 69 wk of age in large flocks in a perchery system and birds preferred to spend more time on perch frame. Channing et al. (2001) focused on the spatial distribution and space usage of perchery housed laying hens at a constant stocking density with colonies of 5 different sizes. Observations began when they were 26 wk of age and continued at 8 wk intervals until 61 wk of age. It was found that older birds spent more time on the floor areas and colony size did not appear to affect the spatial distribution of birds. Pettersson et al. (2018) reported that many factors affected percentage of range use in free-range hens including strains, ages and flock size. Moreover, a previous study demonstrated that the space usage of hens was relatively stable (Odén et al., 2000), and hens only used certain areas at certain times (Pettersson et al., 2016). There were large differences in the space usage of individual hen in the flock (Rufener et al., 2018). Therefore, the identification of individual hen for further spatial distribution and space usage is necessary.

In recent years, individual identification technologies such as machine vision, radio frequency identification, and acceleration sensors have been applied in the field of livestock and poultry breeding (Wang et al., 2019; Chapa et al., 2020; Zanon et al., 2021). Machine vision-based livestock tracking can completely record the trajectory of individual animal, but a multitier system has a small visual space and the birds are obscured from each other, making real-time tracking more difficult. Traulsen et al. (2016) showed that livestock tracking based on accelerometer technology could also be used to record individual trajectories. However, due to the relatively small body size of hens, wearing acceleration sensors necessarily affects the movement of hens between tiers in a multitier system. With the advantages of light weight, small size and easy subregional deployment, RFID technology has been widely used in various applications such as individual hen behavior expression

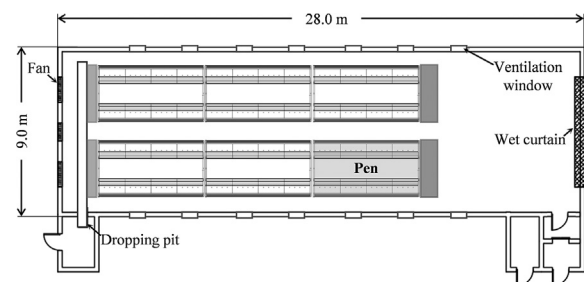
(Li et al., 2017, 2019), activity range (Taylor et al., 2017, 2020), disease monitoring (Roy and Sarkar, 2016), food production, and traceability (Yu and Huang, 2018; Ichiura et al., 2019). The adoption of RFID technology is a more reasonable and easy-to-operate solution for the complete recording of individual hen mobility.

Previous research has reported on egg quality (Molnár et al., 2018; Jabalera et al., 2022), diseases (Buyse et al., 2023), and physical condition (Wall et al., 2022; van Eck et al., 2023) of hens near the end of the laying cycle. Furthermore, the high-intensity physical activity of hens declined with the increasing of age (Kozak et al., 2016) which may lead to the increase of the population size of lower tier in the multitier system, and other issues of bird's health and resources allocation. The objectives of this study were to determine how the vertical space in a multitier system was occupied by hens, and to evaluate the health and welfare of hens near the end of the laying cycle through comparing the spatial distribution, body weight, tibia, body damage, and feather condition of individual birds from different tiers using RFID.

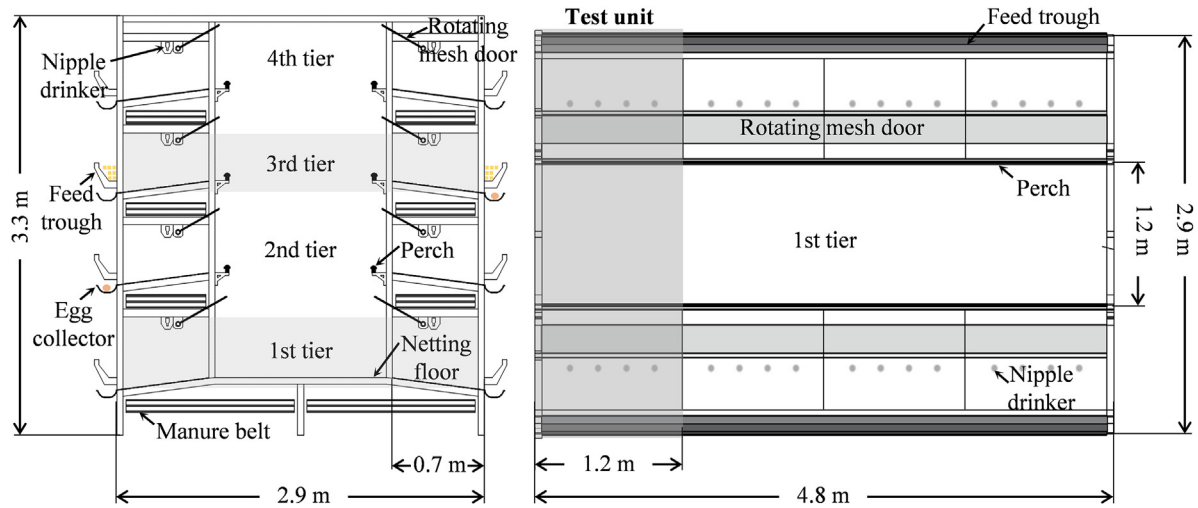
## MATERIALS AND METHODS

### Hens and Housing System

A total of 2,592 Jingfen No. 6 laying hens (Yukou, Beijing, China) were subject to this study in the experimental hen room of the Key Laboratory of Agricultural Engineering in Structure and Environment. The experimental hen room was 28 m long  $\times$  9 m wide. It housed the multitier system with 2 colony rows which were designed and developed by China Agricultural University (Figure 1). Hens were distributed in 2 colony rows with each row divided by wire mesh into 3 pens along the building length. Each pen was 4.8 m long  $\times$  2.9 m wide  $\times$  3.3 m high (Figure 2). This facility had 4 tiers, and each tier was equipped with feed troughs, nipple drinkers, nest boxes with manure belts under each tier. Each tier could be equipped with rotating mesh doors for vaccination management if necessary. Automatic feeding was performed 3 times daily at 7:00 to 8:00, 11:00 to 12:00, and 17:00 to 18:00. Water was provided ad libitum. After 30 wk, the light schedule was 16L:8D (04:30–20:30) with a 30-min dim light period at the beginning and end of the light period. The central egg collection system was used for egg collection once per



**Figure 1.** Top view schematic drawings of the experimental hen room with a multitier system.



**Figure 2.** Side view (left) and top view (right) schematic drawings of 1 pen in the multitier system.

day. Manure was removed every 2 d. The 2nd, 3rd, and 4th tiers were equipped with perches on both inside edge and the length of the perch was 1.2 m. Each pen was initially stocked with 432 hens resulting in a floor space allowance of 917 cm<sup>2</sup>. Temperature, humidity and air quality levels followed commercial management guidelines.

### Flock Distribution During Feeding Time

At 51 and 89 wk of age, the daily flock distribution during feeding time within this multitier system was recorded for 3 consecutive days by manual observation. The number of hens during the 3 feeding periods (7:00, 11:00, and 17:00) in each tier of colony row was counted, and all the recording work had been done within half an hour. Two observers started with either side of the colony row and walked calmly and quietly around both sides to allow for simultaneous counting the number of hens within each tier. Before the start of data collection, observers were trained for 3 d to establish synchrony within observer pairs and ensure a high level of interobserver reliability. Once the number of hens counted of each tier from the 2 observers did not match, the average value was recorded as the number of hens distributed in the tier.

### Focal Hens and Test Unit

In order to further explore mobility of hens near the end of the laying cycle in this system, 12 focal hens at 92 wk of age from each tier were selected randomly during the night and labeled with different RFID tags and number tags (Figure 3). An area in length of 1.2 m in the multitier system was isolated with metal mesh as a test unit for focal hens (Figure 2). Focal hens from 1st tier to 4th tier were sequentially marked in tier-groups labeled as 1 to 4 by tier number. These marked hens were then returned to their original tiers within the test unit. Management remained the same as the whole system.

### Body Weight, Tibia, and Feather Condition

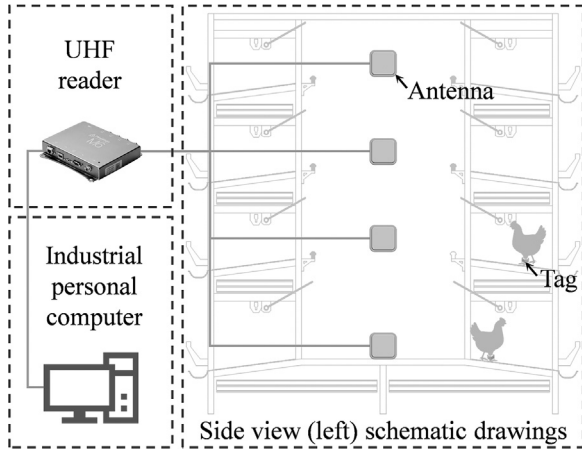
The body weight, tibia size, feather condition, and body damage of focal hens from each tier were measured and compared before transferring to the test unit. Body weight was weighed using a commercial electronic scale (accuracy 10 g) before feeding. Tibia size (length and width) was measured using vernier calipers (accuracy  $\pm 0.03$  mm), where tibia length was the vertical distance from the top of the hock joint to the bottom of the claw, and tibia width was the diameter of the narrowest point in the middle of the tibia. The feathers condition (neck, breast, back, tail, cloaca, and wings), as well as the body damage (rear part of body and comb) were scored after photographing each part individually. Feather condition and body damage scoring referred to the scoring system of Tauson et al. (2005). The system comprised 6 body parts for feather condition (neck, breast, back, tail, cloaca, and wings) and body damage (rear part of body and comb)—all at scores of 1 to 4, with higher scores indicating better integument status. The total score for feather condition and body damage was the sum of the individual scores for each part.

### Radio Frequency Identification Monitoring of the Multi-tier System

A customized ultra-high frequency radio frequency identification (UHF RFID) system was used to monitor the amount time that individual hens spent in each tier and the transition between tiers. Briefly, an



**Figure 3.** RFID tags and number tags attached to the focal hens.



**Figure 4.** A schematic representation showcasing the installation of individual components of the customized ultra-high frequency radio frequency identification (UHF RFID) system.

industrial personal computer (VBOOK-121, Guangzhou Weiwo Electronics Co., Ltd., China), UHF RFID readers (M6, ThingMagic Corporation), antennas (RFA915-9R90-A, Shenzhen Quanshunhong Technology Co., Ltd., China) and tags (TAG915-2918, Shenzhen Quanshunhong Technology Co., Ltd., China) were used to identify and track the individual focal hens.

All 4 antennas (902–960 MHz frequency range, 9 dBi gain, 260 mm long  $\times$  260 mm wide  $\times$  40 mm thick) were positioned at the horizontal midpoint of the metal mesh on the right side of the test unit. The installation height of the antennas closely aligned with the bottom mesh height of each tier. The antennas were situated such that the tag (915 MHz frequency range, 20 mm inner diameter) attached to a hen’s ankle could be registered when the hen was present at the perch. All 4 antennas were connected to a 4-channel reader (902–928 MHz frequency range) that was further connected to the computer via an Ethernet connection (Figure 4).

The data acquisition program was written in Java based on an application programming interface (API) to achieve the connection with the reader, data reading,

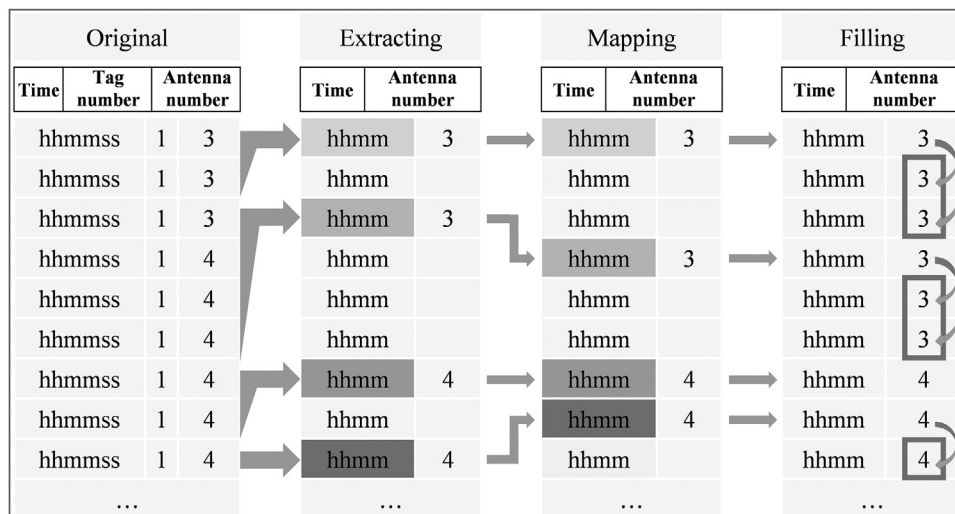
and data recording functions, where the data were stored as CSV files. The table header of the files contained the time, tag number, and antenna number, where the antenna number and the tier number were corresponding.

## Data Processing

After allowing hens 2 d to accommodate to the leg bands and test area, the UHF RFID system was used to continuously collect data of individual focal hens from 5:00 to 16:00 during the day for 6 d. The processing steps of the raw data acquired by the system are shown in Figure 5. In a shorter period of time, the hens did not perform continuous movements between tiers, so the data could be counted by frequency per minute. In the original data, when the number of minutes was the same, only the first data were kept. Since the hens were not always at the perch, the time list of the original data was not continuous. The extracted data were mapped to the full time list by minute. Any missing data points were supplemented with the previous item, that is, it was considered that the hens did not perform movements between tiers. After the above processing, the tier transition data of each hen’s movement between tiers was obtained. The number of hens and time spent in each tier were counted based on the tier transition data. The accumulated occurrences of each antenna number in the tier transition data at giving time were counted as the total number of hens in each tier under that time. The accumulated occurrences of each antenna number in the tier transition data for each focal hen were counted as the time spent on that tier.

## Statistical Analysis

All data were tested for Shapiro-Wilk test (normal distribution test) and  $F$  test (homogeneity of variances test). When the data itself or after transformation (taking the logarithm with a base of 10) did not meet the



**Figure 5.** A schematic representation illustrating the method for extracting, mapping, and filling the raw data collected by the RFID system.

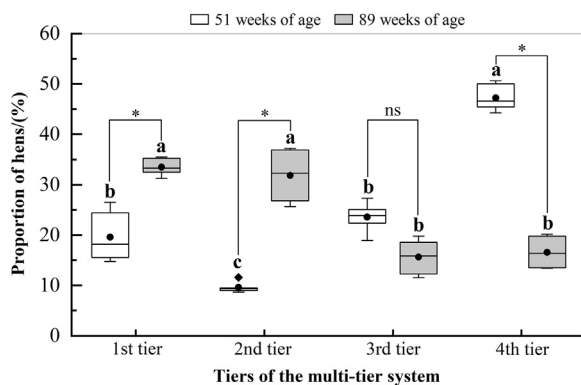


normality requirement, an analysis was conducted using a nonparametric test on the original data. The flock distribution of hens during feeding time was statistically analyzed using the median test for independent samples, and the proportion of time spent, body damage and the feather condition of focal hens from each tier-group in different tiers were analyzed using the independent sample Kruskal-Wallis method. The tibia length, tibia width and body weight of focal hens from each tier-group were analyzed using ANOVA, and those with differences were analyzed using LSD multiple comparison method. All statistical processing was done using SPSS 25.0 statistical software, and graph drawing was done using Origin 2018 drawing software. Data results were expressed as mean  $\pm$  standard deviation, and analytical tests were performed with  $P < 0.05$  as the significance level.

## RESULTS

### Flock Distribution During Feeding Time

The spatial distribution during feeding time of hens at 51 and 89 wk of age in different tiers is shown in Figure 6. The hens were not evenly distributed in the system and varied significantly between the different weeks of age. At 51 wk of age, the flocks were concentrated at the top, with the number of hens in the 4th tier ( $47.3 \pm 2.6\%$ ) being significantly higher than that in the other tiers ( $P < 0.05$ ), while the number of hens in the 1st and 2nd tiers was only  $19.6 \pm 5.0\%$  and  $9.6 \pm 1.1\%$ , respectively. At 89 wk of age, the flocks were concentrated at the bottom, with the number of hens in the 1st ( $33.5 \pm 1.5\%$ ) and 2nd ( $31.9 \pm 5.1\%$ ) tiers significantly higher than that in the 3rd and 4th tiers ( $P < 0.05$ ). The distribution of hens was not fixed with increasing age of the week, but migrated from the top to the bottom. The number of hens in the 4th tier decreased significantly from  $47.3 \pm 2.6\%$  to  $16.6 \pm 3.1\%$  ( $P < 0.05$ ) and in the 3rd tier from  $23.6 \pm 2.9\%$  to  $15.7 \pm 3.4\%$  ( $P > 0.05$ ).



**Figure 6.** Flock spatial distribution during feeding time in different tiers of the multitier system. Different superscripts of lowercase indicate significant difference at the same weeks of age between different tiers ( $P < 0.05$ ). \*Means that there are significant differences at the same tier between different weeks of age ( $P < 0.05$ ). <sup>ns</sup>Means that there are no significant differences at the same tier between different weeks of age ( $P > 0.05$ ).

### Body and Feather Condition of Focal Hens

The results of the body damage and feather condition score for focal hens from 4 tier-groups at 92 wk of age are shown in Table 1. There was no significant difference between the partial scores (rear part of body and comb) and the total score of body damage for focal hens among 4 tier-groups ( $P > 0.05$ ). The total score of body damage was greater than or equal to 7.50 for focal hens among 4 tier-groups.

Overall, there were significant differences in the scores of total feather condition, back and wings among the different tier-groups, which decreased from group 4 to group 1 ( $P < 0.01$ ). The total score of feather condition of hens at 92 wk of age was all below 18 out of 24, and even the group 1 scored only 12. In addition, there was a similar decreasing trend in feather condition scores at the neck, breast, tail and cloaca. Although the trend of feather condition scores fluctuated slightly with decreasing number of tiers, all parts of group 4 were in better feather condition than that of group 1, 2 and 3.

The tibia length, tibia width and body weight measurements of the 4 tier-groups are also shown in Table 1. There was a significant difference in tibia length between the tier-groups, with group 1 having a lower tibia length than that of other groups ( $P < 0.01$ ). There was no significant difference in tibia width and body weight between the tier-groups ( $P > 0.05$ ).

### Mobility and Time Spent of Focal Hens

The number of focal hens in different tiers in the test unit from 5:00 am to 16:00 pm is shown in Figure 7. The number of hens in the 1st and 2nd tiers changed frequently and the changes in numbers were complementary, especially in the 2 feeding periods when there was a general intersection between the number of hens in the 1st and 2nd tiers. All this indicated that the hens interacted frequently between the 1st and 2nd tiers, but less with the 3rd and 4th tiers. The number of hens in the 3rd and 4th tiers remained relatively stable across the day.

The proportion of time spent for focal hens in different tiers is shown in Figure 8. During the observation period, the proportion of time spent in the original tier of tier-groups 1 to 4 was  $91.0 \pm 5.7\%$ ,  $51.9 \pm 5.7\%$ ,  $59.0 \pm 7.0\%$ , and  $63.0 \pm 6.7\%$ , respectively. The proportion of time spent in the original tier was significantly greater in all tier-groups (except the 2nd tier) compared to that of the other tiers ( $P < 0.05$ ). The focal hens from group 2 spent a similar proportion of time in the 1st and 2nd tiers ( $P > 0.05$ ), both significantly greater than the proportion of time spent in the 3rd and 4th tiers ( $P < 0.05$ ).

## DISCUSSION

Hens have a natural habit of roosting in high places (Schrader and Mueller, 2009), with most preferring the top tier of the noncage system. At 51 wk of age, the percentage of the flock in the 4th tier during feeding time

**Table 1.** The body damage, feather condition, tibia size, and body weight of focal hens in different tier-groups.

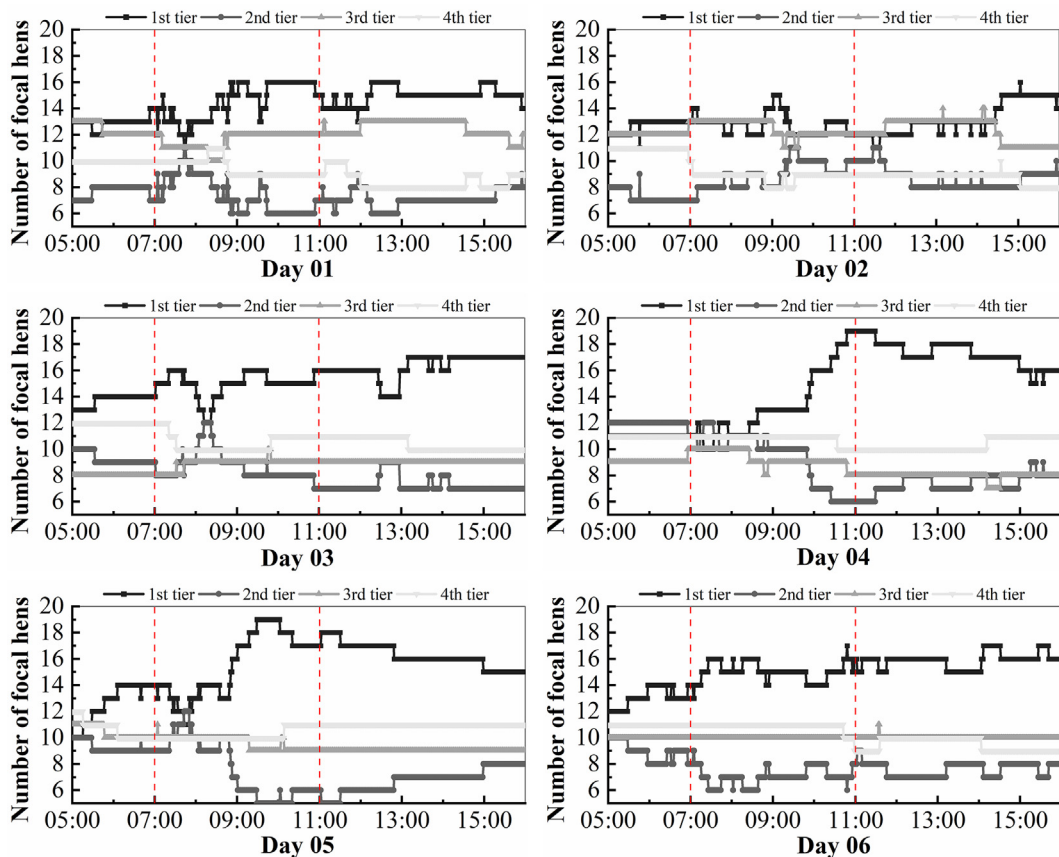
Site		Tier-groups				P value
		Group 1 (n = 12)	Group 2 (n = 12)	Group 3 (n = 12)	Group 4 (n = 12)	
Body damage	Rear part of body	3.83 ± 0.39	3.92 ± 0.29	3.83 ± 0.58	3.83 ± 0.58	0.914
	Comb	3.67 ± 0.49	3.75 ± 0.62	3.67 ± 0.49	3.75 ± 0.45	0.842
	Total score	7.50 ± 0.67	7.67 ± 0.65	7.50 ± 0.90	7.58 ± 0.67	0.896
Feather condition	Neck	2.50 ± 0.67	2.83 ± 0.58	2.67 ± 0.49	3.08 ± 0.67	0.129
	Breast	1.92 ± 1.00	2.42 ± 0.90	2.42 ± 1.00	3.00 ± 0.95	0.071
	Back	1.58 ± 1.00 <sup>A</sup>	1.92 ± 0.51 <sup>AB</sup>	2.42 ± 0.90 <sup>AB</sup>	2.75 ± 0.87 <sup>B</sup>	0.005
	Tail	1.58 ± 1.00	1.50 ± 0.67	2.00 ± 1.13	2.50 ± 1.17	0.091
	Cloaca	2.25 ± 0.75	2.58 ± 0.51	2.83 ± 0.94	3.00 ± 0.85	0.140
	Wings	2.17 ± 0.58 <sup>A</sup>	2.67 ± 0.49 <sup>AB</sup>	3.33 ± 0.49 <sup>B</sup>	3.33 ± 0.49 <sup>B</sup>	0.000
	Total score	12.00 ± 4.37 <sup>A</sup>	13.92 ± 2.43 <sup>AB</sup>	15.67 ± 4.23 <sup>AB</sup>	17.67 ± 3.85 <sup>B</sup>	0.006
Tibia size	Tibia length (mm)	94.23 ± 3.33 <sup>A</sup>	97.82 ± 1.59 <sup>B</sup>	96.05 ± 1.26 <sup>B</sup>	96.59 ± 1.99 <sup>B</sup>	0.002
	Tibia width (mm)	8.23 ± 0.50	8.08 ± 0.27	8.07 ± 0.32	8.02 ± 0.27	0.496
	Weight	Body weight (kg)	1.72 ± 0.13	1.83 ± 0.11	1.77 ± 0.16	1.73 ± 0.16

Different superscripts of uppercase letters in the same line indicate significant difference of  $P < 0.01$ .

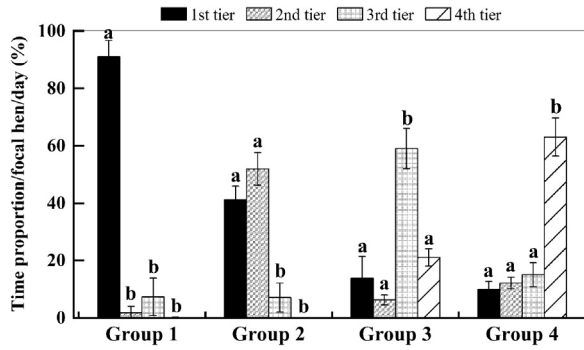
Six body parts for feather condition and body damage—all at scores of 1 to 4, with higher scores indicating better integument status.

was  $47.3 \pm 2.6\%$ , which was about half of the total flock and led to a deviation from the planned standards in terms of the actual stocking density and resource allocation in current multitier system. Nannoni et al. (2022) collected data on the egg distribution of laying hens in combi aviaries at ages ranging from 32 to 78 wk (with data collection occurring every 16 wk). The results indicated that nearly half of the hens exhibited a strong tendency to lay eggs in the higher tier (across the entire trial, 46.9% of the eggs were laid in the top tier, 37.5% in the middle one and 15.6% in the lower one). Other studies have shown that high flock density was particularly

noticeable in upper tiers at 25 to 28 wk of age, exceeding the combined capacity for the upper tiers in an enclosed aviary system (Ali et al., 2016). Overcrowding and pockets of high local stocking densities exist at certain times in the aviary system, and can lead to smothering if not managed appropriately (Arnould et al., 2001). Therefore, some adjustment were needed to improve this situation. With increasing age, the spatial distribution of hens gradually changed from the top to the bottom. The proportion of birds in the 4th tier decreased significantly from  $47.3 \pm 2.6\%$  at 51 wk to  $16.6 \pm 3.1\%$  at 89 wk of age, while the 1st tier increased significantly from



**Figure 7.** Dynamic change of focal hens with RFID tag in the different tiers of the test unit during the day. The red dotted line indicates the start time of feeding.



**Figure 8.** Time spent of focal hens with RFID tag in the different tiers of the test unit. Different superscripts of lowercase in the same column indicate significant difference between different tier-groups ( $P < 0.05$ ).

19.6 ± 5.0% to 33.5 ± 1.5%. The overcrowding situation improved, thereby mitigating the potential negative effects of overcrowding. This indicates that the multitier system in current study is capable of ensuring equal access to recourses which were offered in each tier and a satisfactory level of welfare for laying hens near the end of the laying cycle. Other studies have also shown that the spatial distribution of hens was not fixed, but changed gradually with age (Hegelund et al., 2010; Sibanda et al., 2020). It was observed that birds in the bottom tier had upward exploratory behavior, but were all pecked aggressively by hens in the top tier and forced to return to the bottom tier. At the same time individual animal movement patterns could be influenced by group movement and might further exacerbate flock shifts to the bottom (Gómez et al., 2022). Ali et al. (2016) conducted a spatial distribution study of 4 strains of laying hens at the age of 28 wk and their results showed that the time of the birds roosted in the upper tiers was different. In addition, the number of hens on floor increased over time, because of litter offered on the floor. Overall time spent of ISA Brown hens from 20 wk of age until 69 wk of age in specific area was 47% on the perch frame, 23% on litter, 17% on slatted floor and 9% on nest box in the perchery system (Carmichael et al., 1999). Some studies have shown that keel damage may increase with increasing age, causing pain and limiting the movement of hens (Nasr et al., 2012; Rentsch et al., 2019; Rufener et al., 2019). The current study showed that focal hens at 92 wk spent more time in their original tiers, which was significantly higher than the average time spent in other tiers. This indicated that hens near the end of the laying cycle were mainly active in the same tier, with reduced movement among tiers. Previous research has showed that high-ranking birds used more of the space usage than low-ranking birds (Odén et al., 2004). The average time spent of focal hens from group 1 and group 4 in the original tier was 10.01 h and 6.93 h, respectively, and the results of present study were greater than the residence time of 7.29 h and 4.29 h in the bottom and top tiers reported by Sibanda et al. (2020). Therefore, the strains and the structural layout of the housing equipment may have

led to different spatial distribution and mobility of individual hen.

Focal hens from 1st tier had lower feather scores on wings and back, and shorter tibia length compared to other tiers at 92 wk of age. The total feather condition score for the 1st tier of hens was only 12, which indicates that most of the body feathers were severely damaged (Tauson et al., 2005). Nannoni et al. (2022) similarly indicated that worse plumage condition was found in birds with more freedom to move vertically in combi aviaries as time progressed. In another study on long-life layers, birds kept in a traditional floor housing system had a total feather score of less than 12 at 100 wk of age (Wall et al., 2022). As the age of the birds increased, the condition of the hens' plumage deteriorated (Sokołowicz et al., 2023). Poor feather cover may be more evident on long-life layers. In addition, the social hierarchy in birds is measured using the "pecking order" concept. Forkman and Haskell (2004) investigated the social hierarchy within a group of 6 mature hens, and their findings revealed that social hierarchy was predominantly determined by the outcomes of initial confrontations among individuals, thus indicating a relatively stable social hierarchy. However, Grethen et al. (2023) conducted a research involving groups of 20 and 120 hens. Their results indicated that winning success during the young period did not directly predict high rank during the mature period, suggesting that the social hierarchy possesses a degree of fluidity. While smaller groups of hens exhibited a stable social structure, larger assemblies of these hens may experience instability in their social order, potentially leading to welfare concerns. Karaagac et al. (2003) indicated a significantly higher incidence of feather pecking in the unstable rank-order group compared to the stable rank-order group. Therefore, feather pecking could be more pronounced in the multitier system. Our findings of poor feather coverage of birds in the 1st tier were consistent with observations of Freire et al. (2003). Their study also indicated that the hens in the bottom were the ones attacked in the pecking. Therefore, the hens in the bottom were likely to be on the weaker side and that the bottom space may have allowed them to avoid injuries caused by the pecking. The rearing management manual specified a tibia length standard of 96 mm for hens at the 18 wk of age, and apart from the 1st tier, the average tibia length in the other tiers met the prescribed tibia length standard in current study. The study by Kolakshyapati et al. (2019) demonstrated that the use of range had no influence on tibial parameters. The research conducted by Anderson and Adams (1994) further affirmed that tibial length was independent of both rearing methods and floor types. Meng et al. (2017) reported a similar finding, indicating that the type of cage had no influence on tibial length. Prior research had demonstrated that tibial characteristics were related to factors such as breeds (Kraus et al., 2022), feed intake (Forgiarini et al., 2022), and stocking density (Pereira et al., 2021). Individual differences in the flock and early skeletal development may be the reason for the substandard tibial lengths of hens in the 1st tier.



According to the flock spatial distribution during feeding time, the number of birds was decreased in the higher tiers and increased significantly in the lower tiers with increasing age. This suggested that more weaker birds moved down and may avoid feather pecking from stronger birds in upper tiers. It was also observed that hens in the 1st and 2nd tiers rarely visited the higher tiers based on the time spent in different tiers. A previous study demonstrated that the hens in the bottom were the ones attacked in the pecking (Freire et al., 2003). Hence, it was suspected that the weaker hens can avoid the hens from higher tiers by staying in the bottom, which could dramatically reduce the injuries caused by the aggressive behavior in the current system. Furthermore, focal hens from 1st tier had no difference in body damage, tibia width and body weight with hens from other tier-groups. This also proved that birds that occupied in 1st tier had equal access to feeding resource and did not affect their body weight, which was good for their welfare and health in current system.

## CONCLUSIONS

With the increasing age, the distribution of the flock changed from more hens occupying upper tiers to a more even distribution among all levels of the multitier system. Focal hens from lower tiers in the system did not move among all tiers as much as focal hens from higher tiers in the late laying period. Together the flock level distribution data along with information on movement and condition of focal hens suggested that some hens in this multitier system used fewer tiers as they aged but that this may have allowed hens that were weaker or of lower rank to avoid aggressive behavior from other birds. Feather condition disparities were predominantly concentrated on the back and wings, with feathers of hens in the upper 2 tiers exhibiting better condition than feathers of hens in the lower 2 tiers. However, there were no observed differences in body damage, weight, and tibia width among hens from different tiers. The welfare and health for end of laying hens was maintained at a satisfactory level in this multitier system with equal resources placed in different tiers.

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## DISCLOSURES

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

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