



Article

Effects of Body-Mounted Inertial Measurement Unit (IMU) Backpacks on Space Use and Behaviors of Laying Hens in a Perchery System

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Abstract: Body-mounted sensors have significantly enhanced our understanding of individual animals through location tracking, behavior monitoring, and activity determination. However, attaching sensors may alter the behavior of the tested animals, which would, potentially, invalidate the collected data. The objective of this study was to evaluate the effects of wearable backpacks on space use (feeder, nest box, and perch) and behaviors (aggressive, comfort, and locomotion behaviors) of laying hens in a perchery system. Nineteen laying hens were reared for 21 days, and each was fitted with a lightweight inertial measurement unit (IMU) backpack on day 0. Instantaneous scan samples were adopted to record the number of laying hens, using each space at a 5-min interval over the 16 h lights-on period at −6 d to −1 d, 1 d to 4 d, and 10 d to 15 d. Six hens were randomly selected for observation of behaviors during six 20-min periods at −5 d to −3 d, and 13 d to 15 d. Feeder use reduced at 1 d to 4 d, 11 d, and 13 d to 15 d, and nest box use reduced at 1 d, 3 d, and 10 d to 12 d, while it increased on 15 d. Hens perched more often at 1 d to 4 d and 10 d to 14 d. Space use was affected by wearing a backpack in the first few days after installation. As hens gradually accustomed to the devices, the effects on feeder, nest box, and perch use disappeared at 10 d, 13 d and 15 d, respectively. The diurnal pattern of hens using the nest box largely returned to the state before being backpacked, and there were slight recoveries in the use of feeder and perch use during the 15-day trial period. There was no observed difference in the amount of pecking, preening bouts, aerial ascent/descent, or the time spent on preening and walking at −5 d to −3 d and 13 d to 15 d. No differences were found in body weight and plumage condition score between 0 d and 16 d. The results demonstrated that the IMU backpack only had marginal and non-lasting effects on space use and behaviors of laying hens, and it seems suitable for further behavioral research after short-term acclimation. However, when the diurnal pattern serves as the variable of interest, researchers need to re-evaluate the effect of the device on birds, rather than implying there is no effect.

Keywords: body-mounted sensor; inertial measurement unit (IMU); behavior; space use; laying hen; perchery system



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1. Introduction

Animal behavior is an observable and measurable indicator for assessing physiological health, well-being [1], and preferences for housing systems [2,3]. Advanced developments in lightweight and low-cost body-mounted sensor technology offer researchers an opportunity to track animals across space and time with minimal intervention [4]. For the poultry industry in particular, there has been a conversion from conventional cage systems to cage-free systems, which are characterized by larger flock sizes and more complex environments. Many techniques are available to detect the behavior, activity, and location

of individual chickens employing wearable sensors in large groups, including Radio Frequency Identification (RFID) [5–7], Radio Signal Strength (RSS) [8], accelerometers [9,10], and Ultra-Wideband (UWB) [11]. In order to effectively extrapolate data to the larger and unmarked groups, an implicit assumption associated with these techniques is that these devices should not significantly change the natural behaviors of fitted individuals, but this assumption has rarely been tested [12].

The effects of carrying body-mounted sensors on free-living bird species were studied with a harness as the primary method of attachment, and the outcomes revealed an increase in preening [13] and resting, as well as a reduction in foraging [14]. In order to mitigate the effects of wearable devices on bird's behaviors, it is recommended that the weight of the equipment should be less than 5%, and, more recently, 3% of individual's body weight [15]. Nonetheless, discrepant results were reported by Pietz, Krapu, Greenwood, and Lokemoen [14], who found a significant impact on behaviors even when a lighter device was used. The impacts occurred in wild bird species that predominantly walk and fly, and thus may apply to laying hens. However, the results are likely to be different, as domestic poultry are reared in confined and intensive production systems, which are quite distinct from the open fields where birds live.

Few studies have been conducted to assess the effects of body-mounted devices on the behaviors of poultry. It has been reported that slow-growing broiler chickens performed less walking and pecking behavior in the first week after wearing backpacks with sensors [11]. However, laying hens are distinct from broilers in terms of physique and age, and backpacks may trigger disparate implications, which may be more prone to damaging pecking behaviors elicited by the devices. A study with non-caged pre-lay pullets showed that resource use and agonistic interactions appeared to be affected initially by sensor placement, but quickly become habituated to the tags within 2 weeks [8]. Buijs et al. [16] found that the backpack increased preening of laying hens on the day of fitting, while no effect was observed 2–7 days afterwards. In the aforementioned studies on poultry, birds were raised in floor pens configured with only three perches, which may not fulfill the spatial requirements of natural behavioral expression. With the implementation of healthy and efficient husbandry, a three-dimensional perchery system is regarded to significantly improve the health and welfare status of laying hens by appropriately increasing behavioral performances and activity levels, which would lead to sustainable development for layer industry worldwide [17–19]. However, the effects of wearable sensors on behaviors of laying hens reared in a 3D perchery system are not well understood.

According to the behavioral and physical characteristics of laying hens in a 3D perchery system, a miniaturized, lightweight, and easy-to-attach inertial measurement unit (IMU) backpack was developed, which was mounted by elastic loops around the base of the wings. The objective of this study was to assess how carrying IMU backpacks affected laying hens' space use (feeder, nest box, and perch) and behaviors (aggressive behavior, comfort behavior, and locomotion behavior) in a perchery system.

2. Materials and Methods

2.1. Animals and Housing

Laying hens ($n = 19$; Jingfen 6) sourced from Beijing Yukou Poultry Co., Ltd., Beijing, China were transported from a novel aviary system to the tested perchery system designed for this study located at the Experimental Station, China Agricultural University, Beijing, China, at the age of 65 weeks. The perchery system measured $4.5 \times 1.6 \times 2.8$ m (L \times W \times H), and was furnished with a feeder, water nipples, a nest box, perches, and elevated platforms (Figure 1) to allow the birds to perform their natural behaviors inside. In the system space, 12 perches were categorized into three groups based on the height (h) from ground: low perches (*p*-low, $n = 7$; No.1, 2, 3, 4, 9, 10 and 11; $h < 1.0$ m), medium perches (*p*-medium, $n = 3$; No. 5, 6, 12; $1.0 \text{ m} \leq h < 1.5$ m), and high perches (*p*-high, $n = 2$; No.7, $h = 1.8$ m). Two platforms were constructed between the No. 3 and 4 perch, and between the No. 5 and 6 perch, respectively, to facilitate the birds' access to the upper space.

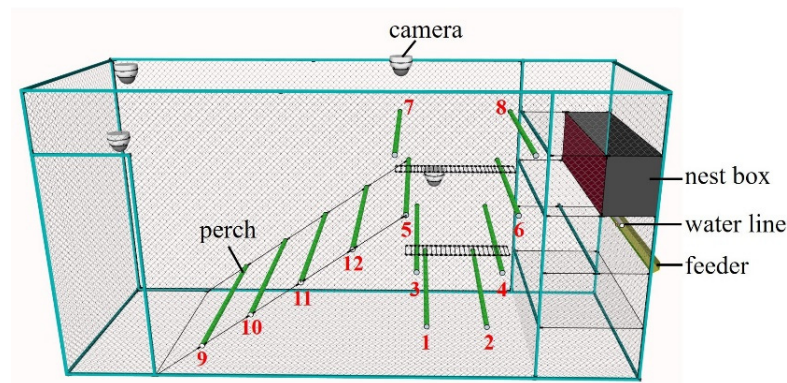


Figure 1. The schematic diagram of the perchery system ($4.5 \times 1.6 \times 2.8$ m (L \times W \times H)). Perches were numbered from 1 to 12 (Height: No. 1, 2: 400 mm; No. 3, 4: 800 mm; No. 5, 6: 1400 mm; No. 7, 8: 1800 mm; No. 9: 280 mm; No. 10: 560 mm; No. 11: 840 mm; No. 12: 1120 mm). Four cameras were fixed to the ceiling, wall, and platform.

Feed and water were offered ad libitum. Daily care was offered at least twice per day, including egg collection, feeding, and hen inspection. The lighting schedule was set to 16L:8D (dark between 21:00 h and 05:00 h). Temperature was maintained between 16°C and 22°C using a ventilation fan and forced air heating. The birds were given more than 40 days to acclimate to the perchery system before data collection. All experimental procedures were approved by the China Agricultural University Experimental Animal Welfare and Animal Experimentation Ethics Committee (Approval ID: AW80702202-5-1).

2.2. IMU Backpack

The body-mounted backpack (Figure 2) consisted of a low-power IMU (LSM6DSL, STMicroelectronics, Switzerland), featuring a tri-axial accelerometer and a tri-axial gyroscope, which were configured to collect acceleration and angular velocity signals at 20 Hz, as well as a microcontroller (CC2530, Texas Instruments Incorporated (TI), Dallas, TX, USA) and a data storage module (SD Card). The sensors were powered by a lightweight 950 mAh Li-ion battery. The device was wrapped in a waterproof heat shrinkable tube casing and attached to the back of the hen, using figure-eight elastic loops around the wing base to construct a backpack. The casing was covered with white label fabric, which was marked with a unique identifying number using black paint to allow for easy identification of individual hens on video recordings. The total weight of the backpack ($45 \times 27 \times 10$ mm) was about 30 g, which was equivalent to approximately 1.7% of hen's body mass.

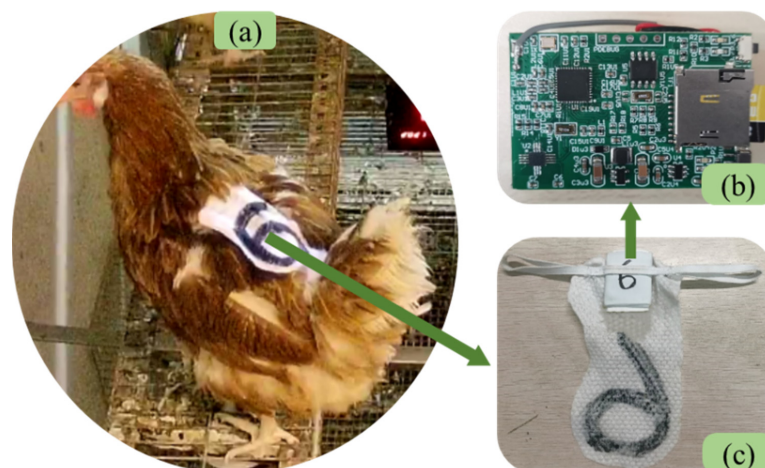


Figure 2. The photograph of (a) a laying hen wearing a backpack, (b) IMU sensors, and (c) the IMU backpack with figure-eight elastic loops.

2.3. Data Collection and Behavior Observation

Four top-view cameras (2DC2402IW-DE3 webcam, Hangzhou Hikvision Digital Technology Co., Ltd., Hangzhou, Zhejiang, China) were fixed to the chamber ceiling and wall, as well as the perchery platform, to record video footage 24 h per day during the trial (Figure 1). Hens were videotaped continuously from −6 d to −1 d before any handling or backpack attachment. The day on which the birds were fitted with backpacks was set as 0 d (65 weeks of bird age). Thereafter, the behavior changes of hens were continuously observed at two stages: right after the backpack placement (1 d to 4 d), and after 10 days of acclimation (10 d to 15 d).

The video was analyzed in order to define the most common behaviors that the hens engaged in the perchery system at the observed time points. Hen behaviors were categorized as space use, aggressive behavior, comfort behavior, and locomotion behavior, as defined in Table 1 [20]. Space use consisted of the hens using the feeder, nest box, and perch. Aggressive behavior involves pecking, and comfort behavior includes preening. Locomotion behavior involves activities of walking and aerial ascent/descent behavior of the birds in the space.

Table 1. Ethogram of observed behaviors in the perchery system.

Behaviors		Description
Space use	Feeder	Hen has head in feeder and is pecking at the feed with beak
	Nest box	Hen is occupying the nest box
	Perch	Hen is on the perches
	Perch-low	Hen is on the low perches
	Perch-medium	Hen is on the medium perches
	Perch-high	Hen is on the high perches
Aggressive behavior	Pecking	A hard, fast stab with the beak at another hen
Comfort behavior	Preening	Moving the beak or bill through the feathers
	Preening bout	Moving the beak or bill through the feathers. A new bout was scored after an interruption of preening of at least 2 s
Locomotion behavior	Walking	Forward movement more than two steps
	Aerial ascent/descent	Jumping or flying between perches, platforms, or other facilities

2.3.1. Space Use

In order to record the space use, instantaneous scan samples were taken at 5-min intervals over the 16 h lights-on period of observation, at −6 d to −1 d, 1 d to 4 d, and 10 d to 15 d. In the test, the number of hens using the feeder, nest box, and perches was counted and divided by the total number of hens in the chamber in order to derive the proportion of hens using each space. During lights-off period, the number of laying hens that rested on the low-, medium-, and high-height perches were also counted, and were then converted to the proportions as described above.

- Similarity measure using Euclidean Distance (ED) score

The Euclidean Distance (ED) measure, one of the simplest similarity measures in the time series, is used to compare the variation in the diurnal pattern of space use on all observation days. By assuming that two time sequences' *C* and *D* vectors are of the same length *n*, and *C_i* and *D_i* are the *i*th values of *C* and *D*, respectively, the ED between time series *C_i* and *D_i* is defined as [21]:

$$d(C, D) = \sqrt{\sum_{i=1}^n (C_i - D_i)^2} \tag{1}$$

ED score can be found by adding 1 to the function and inverting it [22]:

$$s = 1/(1 + d(C, D)) \quad (2)$$

and it returns a value between 0 and 1, where 1 indicates the maximum similarity.

2.3.2. Behaviors

Behaviors were assessed using direct continuous focal observation. Six hens were randomly selected for visual analysis of aggressive behavior, comfort behavior, and locomotion behavior over six 20-min periods during the lights-on period (05:00 h–05:20 h, 08:00–08:20 h, 11:00–11:20 h, 14:00–14:20 h, 17:00–17:20 h, 20:00–20:20 h) at –5 d to –3 d and 13 d to 15 d. Each instance of pecking and aerial ascent/descent and each bout of preening were recorded. The total time engaged in preening bouts, as well as the total time spent walking per 20-min observation session, were also recorded, and they were converted to a percentage of the total observation period for analysis.

2.3.3. Body Weight and Plumage Condition

Individual body weight and plumage damage were assessed on 0 d and 16 d. The plumage condition of the hen's back followed a four-point scoring system (1–4), in which the higher score represented the better status of the integument [23].

2.4. Statistical Analysis

Statistical analyses were performed in SPSS (IBM SPSS Statistics 25, Armonk, NY, USA). Data on the proportion of hens using each space before and after backpack attachment at day level were analyzed using a general linear model (GLM), with the days as the fixed factors. Non-parametric analysis of variance (Kruskal–Wallis H Test) was used to compare the differences in total counts of pecking, aerial/descent, and preening bouts that were aggregated at the day level. Preening and walking times, as a percentage of total observation period at the day level, were also analyzed using the Kruskal–Wallis test. A paired t-test was used to compare the body weight of hens at 0 d and 16 d. Statistical significance was set to $p < 0.05$. Once the significance was observed, post hoc tests were performed with the Least Significant Differences (LSD) criterion. The normality of the residuals was checked visually using P-P plots and histograms. Results were presented as least squares mean \pm standard error (SE).

3. Results

3.1. Body Weight and Plumage Condition

No difference in the body weight of the laying hens was detected before or after backpack attachment (mean \pm SE; 0 d: 1.75 ± 0.11 kg, 16 d: 1.73 ± 0.11 kg, $t = 1.425$, $p > 0.05$). The plumage condition score (≥ 3) on the back of each hen did not change during the experiment; therefore, no statistical analysis was applied.

3.2. Space Use

Figure 3 shows that no differences were found in the percentage of hens using a feeder ($F = 0.217$, $p > 0.05$), nest box ($F = 0.448$, $p > 0.05$), or perch ($F = 0.838$, $p > 0.05$) at –6 d to –1 d. Therefore, data collected from –6 d to –1 d were averaged and used for comparison with each observation day after backpack placement (1 d to 4 d, 10 d to 15 d) in the following statistical analysis. The feeder was less used after backpack placement, but no differences were observed at 10 d and 12 d compared to the days before placement ($p > 0.05$; Figure 3a). Nest box use was reduced after backpack attachment, while it increased at 15 d ($p < 0.05$), and no difference was found at 2 d, 13 d, or 14 d ($p > 0.05$; Figure 3b). The birds perched more often after backpack installation, but no difference was observed at 15 d ($p > 0.05$; Figure 3c).

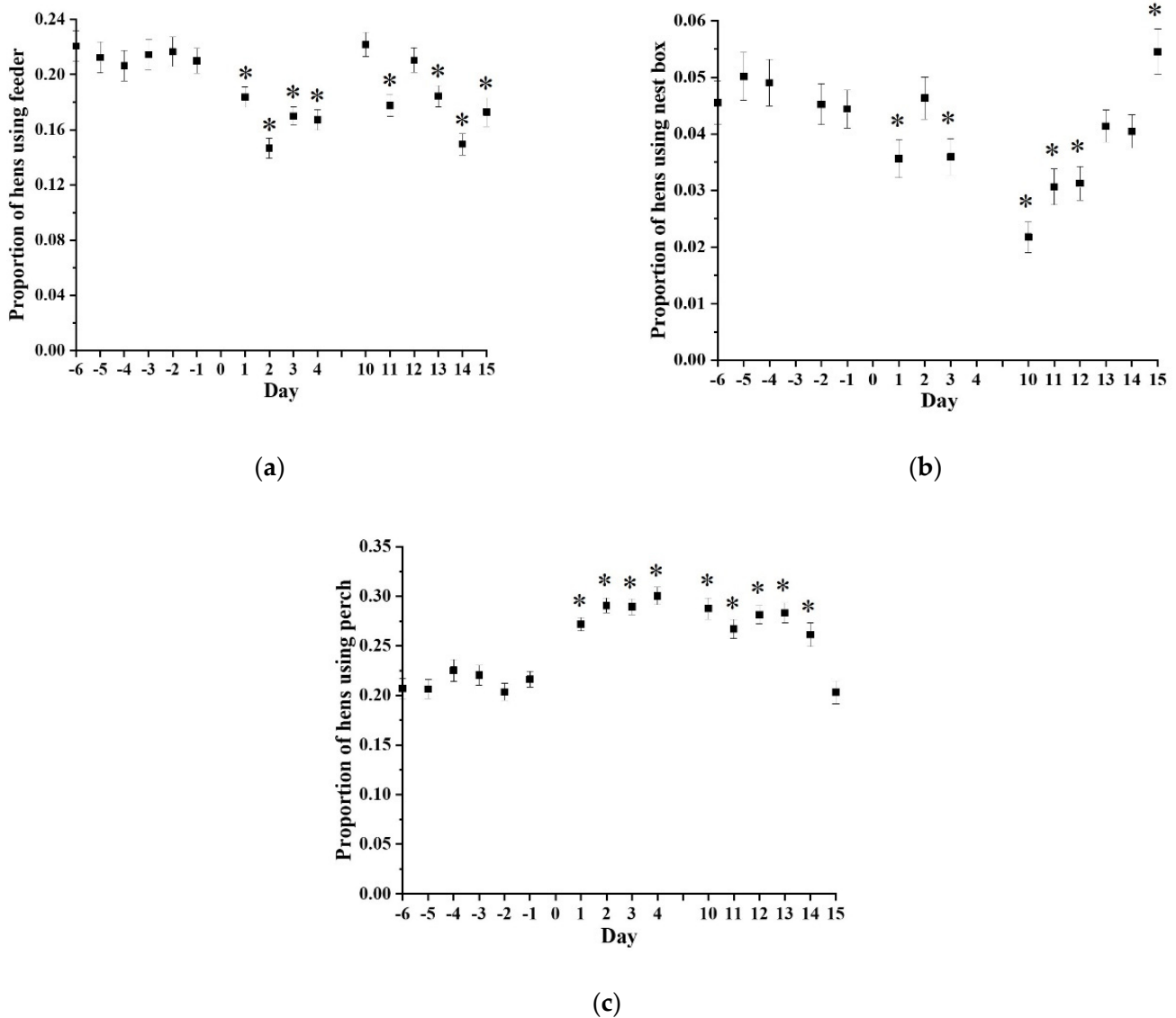


Figure 3. Proportion of laying hens using (a) feeder, (b) nest box, and (c) perch on all observation days. Differences between each observation day after backpack attachment and the mean from -6 d to -1 d ($p < 0.05$) are indicated with an asterisk.

Figure 4 shows the percentage of laying hens using the perches of low-, medium-, and high-height, as well as the overall levels on the observation days from -6 d to -1 d, 1 d to 4 d, and 10 d to 15 d when lights were on (Figure 4a) and off (Figure 4b), respectively. For high-height perches, no differences were observed on observation days (-6 d to -1 d, 1 d to 4 d, and 10 d to 12 d; $p > 0.05$), except at 13 d and 14 d. For medium-height perches, no difference were observed in the proportion of birds on perches at -6 d to -1 d, 3 d, 4 d and 10 d to 15 d ($p > 0.05$) except at 1 d and 2 d. For low-height perches, perch use at 1 d to 14 d was higher than it was on the days before backpack attachment ($p < 0.05$). During the lights-off period, the average proportion of hens using perches overall was $68.42 \pm 1.92\%$ (mean \pm SE), $64.47 \pm 1.32\%$, and $75.49 \pm 1.81\%$ at -6 d to -1 d, 1 d to 4 d, and 10 d to 15 d, respectively. The lower perches were barely used when the birds were sleeping compared with the upper perches (medium and high perches) (Figure 4b).

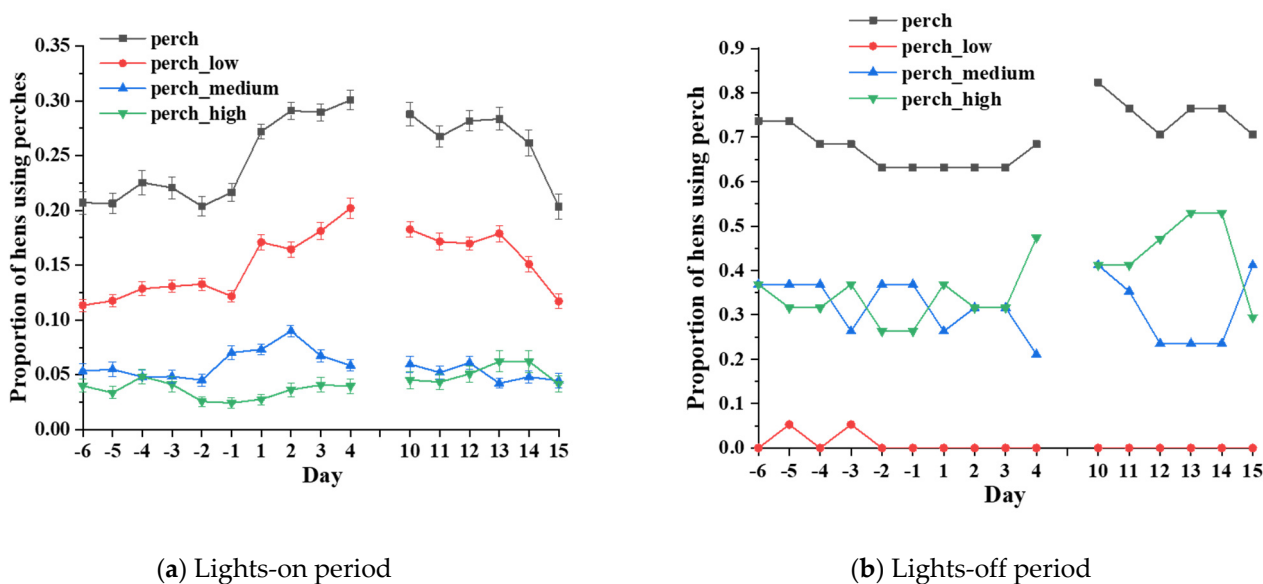


Figure 4. The proportion of laying hens using perches of low-, medium-, high-height, as well as overall levels, on all observation days during the lights-on (a) and lights-off (b) periods.

- Diurnal pattern of space use

Figure 5(a1,b1,c1) show the diurnal pattern of hens using the feeder, nest box, and perch at -6 d to -1 d before backpack attachment, respectively. The ED score was adopted for a similarity measure between each two curves of the diurnal pattern of each measure of space use at -6 d to -1 d. The ED scores for the diurnal pattern of feeder use were greater than 0.700, except at -6 d vs. -1 d (0.694), -4 d vs. -5 d (0.686), and -2 d vs. -5 d (0.686) (Table 2). For nest box use, ED scores were greater than 0.800, except for at -1 d vs. -5 d (0.799) (Table 3). For perch use, ED scores were mostly greater than 0.700, except at -4 d vs. -1 d (0.686) (Table 4). Due to the similarity between the diurnal pattern of each measure of space use before backpack placement, the average percentage of birds using the feeder, nest box, and perch throughout the lights-on period was depicted by thick orange lines, as shown in Figure 5(a1,b1,c1). This was treated as the baseline and used for comparison with the diurnal pattern of space use after backpack placement.

Table 2. The Euclidean Distance (ED) score for the diurnal pattern of hens using the feeder for each day from -6 d to -1 d.

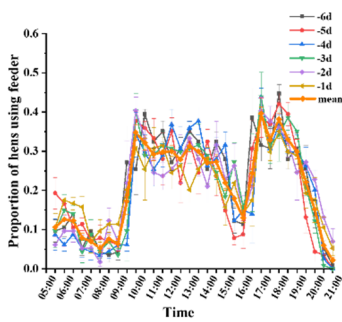
Day	-6 d	-5 d	-4 d	-3 d	-2 d	-1 d
-6 d	1	0.716	0.701	0.704	0.711	0.694
-5 d		1	0.686	0.739	0.686	0.718
-4 d			1	0.756	0.736	0.702
-3 d				1	0.749	0.755
-2 d					1	0.747
-1 d						1

Table 3. The Euclidean Distance (ED) score for the diurnal pattern of hens using the nest box for each day from -6 d to -1 d.

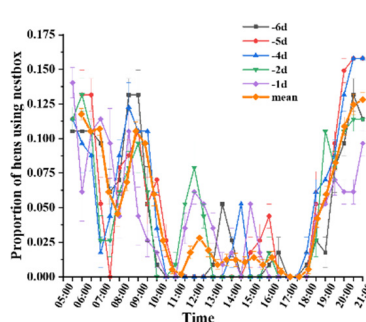
Day	-6 d	-5 d	-4 d	-2 d	-1 d
-6 d	1	0.856	0.862	0.844	0.839
-5 d		1	0.878	0.851	0.799
-4 d			1	0.853	0.802
-2 d				1	0.838
-1 d					1

Table 4. The Euclidean Distance (ED) score for the diurnal pattern of hens using the perch for each day from −6 d to −1 d.

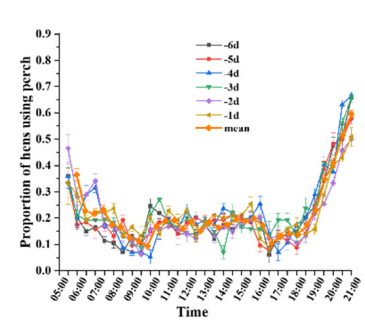
Day	−6 d	−5 d	−4 d	−3 d	−2 d	−1 d
−6 d	1	0.784	0.714	0.782	0.714	0.730
−5 d		1	0.720	0.769	0.713	0.756
−4 d			1	0.724	0.713	0.686
−3 d				1	0.703	0.722
−2 d					1	0.750
−1 d						1



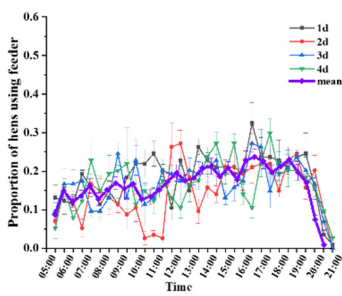
(a1) Feeder use: −6 d to −1 d



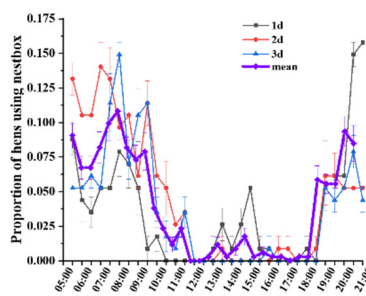
(b1) Nest box use: −6 d to −1 d



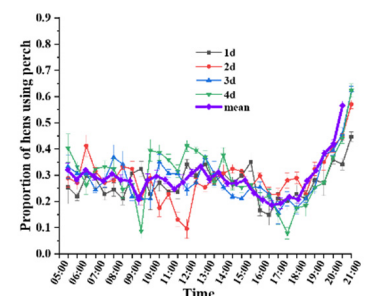
(c1) Perch use: −6 d to −1 d



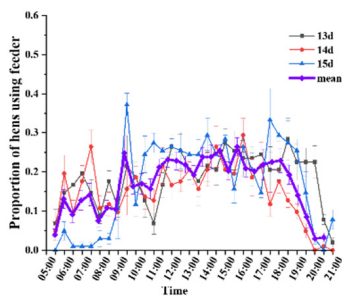
(a2) Feeder use: 1 d to 4 d



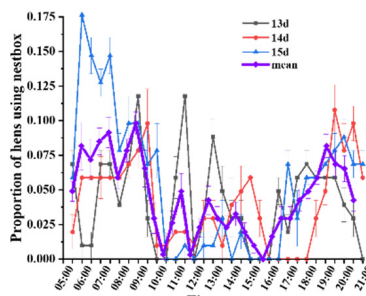
(b2) Nest box use: 1 d to 3 d



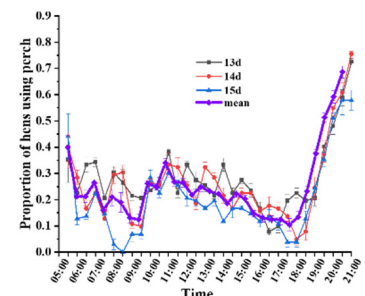
(c2) Perch use: 1 d to 4 d



(a3) Feeder use: 13 d to 15 d



(b3) Nest box use: 13 d to 15 d



(c3) Perch use: 13 d to 15 d

Figure 5. The diurnal pattern of hens using the feeder (−6 d to −1 d (a1), 1 d to 4 d (a2), 13 d–15 d (a3)), nest box (−6 d to −1 d (b1), 1 d to 3 d (b2), 13 d–15 d (b3)), and perch (−6 d to −1 d (c1), 1 d to 4 d (c2), 13 d–15 d (c3)). The average proportions of each measure of space use at −6 d to −1 d were depicted by thick orange lines (baseline), and plotted by thick violet lines at 1 d to 4 d and 10 d to 15 d.

Figure 5(a2,b2,c2), as well as Figure 5(a3,b3,c3), show the diurnal pattern of hens using each space at 1 d to 4 d and 13 d to 15 d. The ED scores for the diurnal pattern of each measure of space use on observation days after backpack placement, as well as the baseline, were shown in Table 5. For feeder use, the ED scores were generally lower than those on the days before equipping. For nest box use, the ED scores at 10 d to 13 d were relatively lower than those on other days after being backpacked. The ED scores for perch use were reduced at 1 d (0.621), dropped to a minimum at 4 d (0.586), and increased to 0.722 at 15 d.

Table 5. The Euclidean Distance (ED) score for the diurnal pattern of hens using the feeder, nest box, and perch on observation days after the backpack placement, as well as the baseline.

ED Score	Feeder Use	Nest box Use	Perch Use
1 d vs. baseline	0.674	0.863	0.621
2 d vs. baseline	0.578	0.832	0.615
3 d vs. baseline	0.645	0.829	0.638
4 d vs. baseline	0.606	—	0.586
10 d vs. baseline	0.622	0.785	0.659
11 d vs. baseline	0.613	0.790	0.666
12 d vs. baseline	0.599	0.798	0.672
13 d vs. baseline	0.643	0.769	0.661
14 d vs. baseline	0.584	0.850	0.671
15 d vs. baseline	0.653	0.824	0.722

3.3. Behaviors

No differences were found in the number of observed instances of pecking ($H = 8.327$; $p > 0.05$), preening bouts ($H = 3.518$; $p > 0.05$), or aerial ascent/descent counts ($H = 6.080$; $p > 0.05$). In addition, no differences were found in the percentage of observation time spent preening ($H = 4.316$; $p > 0.05$) or walking ($H = 6.445$; $p > 0.05$) at -5 d to -3 d, or at 13 d to 15 d, before or after backpack placement.

4. Discussion

Generally, backpack presence had no effects on the body mass or plumage condition on the backs of laying hens in the tested perchery system. Space use and behaviors of laying hens were affected initially by the body-mounted IMU backpack, but only in the first few days after placement, and these effects had almost disappeared by the end of the test, indicating that hens were able to habituate to wearing them. Although the diurnal trend of space use returned to the baseline as the hens acclimated to the new device, the alteration of the diurnal pattern did not completely vanish within the 15-day habituation period.

4.1. Laying Hen Behavior before Backpack Placement

From -6 d to -1 d, there were no differences in the proportion of hens using the feeder, nest box, and perch, or in aggressive, comfort, and locomotion behaviors. The ED scores in Tables 2–4 display that the diurnal pattern of measure of each space use were similar before backpack placement. The feeding peaks were observed at about 10:00 h and 17:00 h on each day. Previous studies documented that laying hens' feeding behavior generally has a diurnal pattern, with a feeding peak in the morning after laying, a decrease in the middle of the day, and an increase towards the evening [24], which is in accordance with our results. The usage of the nest box fluctuated throughout the day from -6 d to -1 d, while the egg-laying peak usually occurred at about 08:30 h (3–4 h after the light was turned on). Laying hens' pre-laying behavior, nesting, and egg laying are closely related to the photoperiod, and the laying behavior generally occurs within 6 h of turning on the lights [25]. The fluctuation in nest box use could be due to the different activities which can be done in the nest box. For instance, hens were observed to rest in the nest box at night and explore it during the lights-on period. A similar diurnal pattern of perch use was also observed from -6 d to -1 d, indicating that the usage of perches was higher just after the

lights were turned on and before the lights were turned off. This was because the birds roosted on the perches at night. Additionally, two valleys of perch use were shown at about 09:30 h and 16:30 h, which were near the feeding peaks. In summary, there were relatively constant diurnal patterns of hens using each space without constraints or disturbances. It is reasonable to assume that hens have developed stable behavioral patterns by 65 weeks of age. Therefore, the changes in behaviors of hens, if any, were only treated as being affected by attaching the backpacks.

4.2. Effect of Backpack on Space Use

Changes in the access to the feeder, nest box, and perch declared that the space use of laying hens was affected at first when wearing the backpacks. Hens spent less time feeding due to discomfort in the backpacks, which is consistent with other studies [8,11]. Dennis, Fahey, and Cheng [12] reported that the tagged chickens were lighter due to decreased access to the feeder, but no statistical reduction in body weight was observed in our test. An initial reduction in nest box use by hens after backpack placement was observed. The nest box was constructed at a height of 1.4 m, requiring the hens to use a complex route in order to access it, which may have partially accounted for the reduction, since birds were not yet moving flexibly after equipping. A behavioral observation also revealed that some hens laid eggs on platforms or bottom nets rather than in the nest box. The small number of such hens using the nest box could greatly influence the results. The increased use of perches by hens in the first few days may be due to the restricted motion and perceived change in their mobility, which would cause them to isolate themselves from conspecifics in order to become accommodated to the new devices. Even though the backpack was lightweight, hens may still require more operating time to maintain their balance on perches due to the change in body weight distribution [26].

When considering the usage of perches of various heights, hens scarcely stood or rested on the high perch whether or not they were mounted with backpacks during the lights-on period. Within 1–2 days after backpack attachment, hens spent more time on the medium perches, but the difference vanished from the third day onward. The low perch use was in line with that of the total use of the perches during the lights-on period. During the lights-off period, the hens slept mainly on upper perches, rarely roosting on low perches. The average proportion of perch use during the night was about 75.5% from 10 d to 15 d, which showed no reduction compared with –6 d to –1 d. A similar ratio (76–80%) was reported by Appleby et al. [27]. This indicates that the backpack had no effect on the resting habits of hens. Summarily, hens tended to use lower perches during the lights-on period and higher perches at night, which agrees with the perching pattern found in other studies [28,29].

The similarity of the diurnal pattern of each measure of space use to the baselines decreased to varying degrees after backpack placement. The previously discernible feeding peaks of hens before equipping disappeared when newly backpacked, and the proportion of feeder use was essentially maintained within a certain range, accompanied by slight fluctuations without apparent peaks throughout the day (Figure 5(a2)). This indicates that the diurnal pattern of feeder use was disrupted by wearing the backpacks. Changes were also observed in the perch use, where the troughs became less distinct in the first few days after being backpacked compared to before (Figure 5(c2)). It was readily interpreted that in the natural state, i.e., before fitting equipment, the feeding peaks of hens were virtually synchronized with the onset of the valleys of perch use. For the usage of the nest box, the peak occurred at almost the same time as before (Figure 5(b2)), suggesting that there was a minor effect on the diurnal pattern of nest box use.

As hens gradually became accustomed to the new backpacks, the proportion of birds using the feeder, nest box, and perch returned to similar levels compared to that before placement at 10 d, 13 d, and 15 d, respectively, but still fluctuated slightly. Carmichael, Walker, and Hughes [25] have found a linear increase in the nest box use of laying hens, even after the egg production peak, in an aviary system. Therefore, the increased number of

birds approaching the nest box at 15 d did not mean that wearing backpacks would have an adverse impact on hens' production. Previous studies have illustrated that the birds became accustomed to body-mounted devices over a period of days to weeks [13,30,31], which is in accordance with our findings. Conversely, several studies suggested that behavioral alteration caused by wearing device did not disappear over time [32,33]. In this study, the diurnal pattern of nest box use largely returned to the level before backpack placement, but there were slight recoveries in the levels of feeder and perch use during the 15-day trial period.

4.3. Effect of Backpack on Behaviors

4.3.1. Aggressive Behavior

No increase in levels of aggressive pecking was observed after backpack attachment. The result suggested that backpack presence did not stimulate or increase the aggression of hens caused by the discomfort of wearing devices [34]. There was a more distinct increase in aggression due to the higher chance of encounters between individuals when hens could not access food, or during food deprivation [35]. Sufficient feeder space was provided in this study, which could partially explain the lack of an increased number of incidents of aggression. The hens were more likely to isolate themselves from conspecifics when wearing new devices, thus reducing the opportunity for confrontation. It has been observed that the effect of marking diminishes or disappears if a larger percentage of the birds in the group are marked [36,37]. All birds were mounted with backpacks in this study, which made it less likely that the birds would increase their aggressive behaviors.

Body-mounted devices may alter the aggressive interactions of animals by changing their appearances. A study with non-caged laying hens found that carrying sensors have no effect on agonistic interactions, possibly because the sensor case and harness were colored to blend in with the hen's feathers, and thus did not attract the attention of other birds [8]. Previous studies with broilers illustrated that individuals displayed more aggression with markers on the head and neck [37]. Another study on laying hens demonstrated that the wing and leg band identification systems may increase feather pecking, but no effect was reported for tail marking [12]. In the current study, the backpack had no effect on the aggressive behaviors, which is likely because the back is not evolutionarily important for propagating the signal status of hens. The research on broilers with similar backpacks to the present study found that there were more pecks only in the first week after backpack placement, which could be classified as 'exploratory,' but the difference was no longer detected from the second week onwards. This indicated that the birds had explored the novel devices and had become less interested in them upon repeated exposure [11].

4.3.2. Comfort Behavior

It was supported by the informal observation that hens were spending more time on preening only during the first few days after the placement, which was consistent with previous studies. Harness and backpack attachments have been shown to increase the rates of and time spent preening [38]. The study on broilers showed that the number of preening bouts was higher in the first week after backpack attachment due to the novelty of the devices [11]. However, increased preening disappeared quickly, indicating that birds were no longer curious or uncomfortable with the backpacks after acclimation.

4.3.3. Locomotion Behavior

The backpack had limited effects on the locomotion behaviors, including walking and aerial ascent/descent of the hens. Informal observation suggested that hens may have initially spent less time walking after backpack attachment. Similar results were reported by Stadig, Rodenburg, Ampe, Reubens, and Tuytens [11], who observed that broilers walked less only in the first week after sensor placement, after which no further differences were found. Birds often showed freezing behavior and difficulty walking initially after fitting, even when birds carried empty backpacks [39]. In this study, hens were observed walking, aerially ascending/descending, and accessing all space seemingly without difficulty after

the habituation period, indicating that the backpack did not affect movement, agility, or ability to perform behaviors in laying hens.

5. Conclusions

The body-mounted IMU backpack had relatively mild and non-persistent effects on space use, aggressive behavior, comfort behavior, and locomotion behavior of laying hens in a perchery system. Backpack presence reduced the feeder and nest box use but increased perch use only in the first few days after placement, and the effects had almost disappeared by the end of the test, indicating that hens were able to habituate to wearing the backpacks. These findings can also be extrapolated to other types of body-mounted sensors or markings with similar or smaller dimensions and lower weight, as well as similar attachment methods for laying hens. The alteration of the diurnal pattern of space use, although it had a tendency to recover, did not disappear entirely within 15 days of habituation. Further work may highlight whether space use could return to original levels and patterns with stability when hens take more time for habituation. In future studies, trade-offs should be made between these negative effects and the valuable benefits obtained through body-mounted devices.

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