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Animal welfare in free-range systems: Integrating environmental, behavioral, and physiological aspects in Lohmann Brown egg-laying hens

Bem-estar animal em sistemas de criação ao ar livre: Integrando aspectos ambientais, comportamentais e fisiológicos em galinhas poedeiras Lohmann Brown

Bienestar animal en sistemas de cría al aire libre: Integración de aspectos ambientales, conductuales y fisiológicos en gallinas ponedoras Lohmann Brown

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Abstract
The free-range system has been used as an alternative to promote the welfare of laying hens. This study aimed to evaluate the environmental and behavioral factors of laying hens raised in this system. Conducted in a commercial barn, the study involved hens aged 18-24 weeks from the Lohmann-Brown lineage. The microclimate, aviary bedding, and nests were assessed, along with pododermatitis and feather condition. Measurements of temperature, respiratory frequency, and corticosterone hormone analysis were also conducted. The climatic conditions were favorable, remaining within the thermoneutral zone, with no incidence of pododermatitis or feather lesions. Natural, social, feeding, reproductive, stress-related, locomotor, exploratory, and resting behaviors were observed, with morning highlighting internal area and afternoon external area. Maintenance behaviors prevailed in the afternoon, with greater social behavioral expression in the intermediate period. The interaction between observation period and age of the hens had a significant effect on body temperature. Behavioral and physiological assessments are effective tools for evaluating animal welfare. It is concluded that the free-range system is satisfactory in terms of comfort and welfare of laying hens.
Keywords: Behavior; Corticosterone; Health; Physiology; Thermal comfort.

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Resumo
O sistema free-range tem sido utilizado como alternativa para promover o bem-estar de galinhas poedeiras. O objetivo deste estudo foi avaliar os fatores ambientais e comportamentais de galinhas poedeiras criadas neste sistema. Realizado em um galpão comercial, o estudo envolveu galinhas de 18 a 24 semanas da linhagem Lohmann-Brown. Foram avaliados o microclima, a cama do aviário e os ninhos, além da pododermatite e condição das penas. Também foram realizadas medições de temperatura, frequência respiratória e análise do hormônio corticosterona. As condições climáticas foram favoráveis, permanecendo dentro da zona termoneutra, sem incidência de pododermatite ou lesões nas penas. Foram observados comportamentos naturais, sociais, alimentares, reprodutivos, relacionados ao estresse, locomotores, exploratórios e de repouso, com destaque para a área interna pela manhã e para a área externa à tarde. Os comportamentos de manutenção prevaleceram no período da tarde, com maior expressão comportamental social no período intermediário. A interação entre o período de observação e a idade das galinhas teve efeito significativo na temperatura corporal. As avaliações comportamentais e fisiológicas são ferramentas eficazes para avaliar o bem-estar animal. Conclui-se que o sistema free-range é satisfatório em termos de conforto e bem-estar de galinhas poedeiras.
Palavras-chave: Comportamento; Corticosterona; Saúde; Fisiologia; Conforto térmico.

Resumen
El sistema free-range ha sido utilizado como alternativa para promover el bienestar de gallinas ponedoras. El objetivo de este estudio fue evaluar los factores ambientales y conductuales de gallinas ponedoras criadas en este sistema. Realizado en un galpón comercial, el estudio involucró gallinas de 18 a 24 semanas de la línea Lohmann-Brown. Se evaluaron el microclima, la cama del aviario y los nidos, además de la pododermatitis y la condición de las plumas. También se realizaron mediciones de temperatura, frecuencia respiratoria y análisis de la hormona corticosterona. Las condiciones climáticas fueron favorables, manteniéndose dentro de la zona termoneutra, sin incidencia de pododermatitis o lesiones en las plumas. Se observaron comportamientos naturales, sociales, alimenticios, reproductivos, relacionados al estrés, locomotores, exploratorios y de reposo, con mayor actividad en el área interior durante la mañana y en el área exterior durante la tarde. Los comportamientos de mantenimiento prevalecieron en el período de la tarde, con mayor expresión conductual social en el período intermedio. La interacción entre el período de observación y la edad de las gallinas tuvo un efecto significativo en la temperatura corporal. Las evaluaciones conductuales y fisiológicas son herramientas eficaces para evaluar el bienestar animal. Se concluye que el sistema free-range es satisfactorio en términos de confort y bienestar de gallinas ponedoras.
Palabras clave: Comportamiento; Corticosterona; Salud; Fisiología; Confort térmico.

1. Introduction

The welfare of production animals is becoming increasingly important (Campbell et al., 2020). An example is regarding egg consumers, who in recent years have been seeking those produced in alternative production systems that promote animal welfare (Garcia et al. 2023). Therefore, one of the factors that affects welfare is the environment in which the animals are housed. The environment is the space made up of a physical environment and, at the same time, a psychological environment prepared for the exercise of the activities of the animals (Broom, 2011), and the analysis of its characteristics is extremely relevant.

Among environmental factors, thermal conditions—such as temperature, relative humidity, airspeed, and radiation—directly influence a bird's ability to maintain homeothermy (Olanrewaju et al., 2010) and, under optimal conditions, can enhance productivity. However, when these conditions are not favorable, the environment may induce heat stress (Sánchez-Casanova et al., 2020), prompting birds to make physiological adjustments to maintain a stable body temperature. Studies have shown that facilities in Brazil may not provide optimal conditions for birds in various regions of the country (Bueno et al., 2018; Bonfim et al., 2024).

Animal health and behavior are important considerations when assessing welfare, which depends on providing animals with a good environment (He et al., 2022). The fact that an animal shows changes in its behavior is an indication of its state of comfort and, consequently, its well-being (Broom & Molento 2004).

In cage systems, the limited space restricts the laying hen's ability to perform important natural behaviors, which can increase abnormal behaviors (Janczak et al., 2015), layers raised in alternative systems has shown better feather conditions in back, neck and wings in different ages when compared to the cage systems (Jeon et al. 2025). In this context, the free-range system results in welfare benefits for laying hens (Bari et al., 2020), as the birds are raised freely and in direct contact with the ground (Sibanda et al. 2020), promoting direct and indirect benefits by allowing the animal to perform natural behavioral patterns. In this system, chickens are housed in sheds that provide voluntary access to outdoor areas (Martínez-Pérez et al., 2017). In view of the above, this study aimed to evaluate the environmental and behavioral factors of laying hens raised in this system.

2. Methodology

2.1 Experiment location and type of installation

The research was carried out in a commercial farm with laying hens, located in the municipality of Ipeúna - São Paulo, with a southern latitude of 22°26'09", a western longitude of 47°43'08" and an average altitude of 635 meters. According to the Köppen classification, the climate is Cfa (humid subtropical), with an average annual temperature of 18.1°C and 1,500 mm of accumulated annual rainfall.

The orientation of the commercial barn (Figure 1) is southeast-northwest (124° SE and 310° NW). The experimental period was during the months of July, August, and September 2015, during the pre-laying and laying phases (18 to 24 weeks of age). The facilities, as well as the management, complied with the current standards established by Humane Farm Animal Care (HFAC), which is a certification protocol awarded to production companies that implement and follow standards related to animal welfare, ensuring a balanced diet, the absence of antibiotics, the presence of shelters and resting areas so that the animals can manifest the natural behavior of their species.

Figure 1 - Front (A), rear (B) and interior (C) and (D) views of the commercial free-range layer house.

Source: Research data.

The characteristics of the shed are as follows: Dimensions: 9.85 m wide x 70.0 m long and a ceiling height of 3.00 m; roof with fiber cement tiles, 0.60 m eaves and masonry walls - 60 cm high, with metal mesh and manually operated side curtains; equipped with fans and automatic misting system; concrete floor covered with wood shavings (8 cm high) and nests distributed along the length of the shed in a ratio of 1:6 laying birds and perches; automatic trough-type feeders and pendulum drinkers; picket area (pasture) and trees all around, fenced with wire mesh.

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2.2 Animals used in research and animal handling

The research was submitted to the Ethics Committee for the Use of Animals (CEUA) under protocol number 2015- 09. The day-old chicks were obtained from the hatchery of Hygen Genética Avícola Ltda, Rio Claro/SP. 5,098 laying birds of the Lohmann-Brown strain were housed at a density of 7.28 birds/m2. Water and food were provided ad libitum during the experimental period. In addition, the birds were allowed to run freely in the house and had free access to the paddock.

2.3 Research Stages

2.3.1 Assessment of the microclimate in the facility

Temperature (T, °C), relative humidity (RH, %), and dew point temperature (Tdp, °C) were recorded daily at one-hour intervals. HOBO® thermo-hygrometers, model H08-00X-02, were used, installed at a height of 1.5 meters above the bedding, at three different points inside the shed (beginning, middle and end) and another outside.

For the calculation of enthalpy (equation 1), the evaluation of thermal comfort was based on the range of 50.0 to 68.8 kJ/kg of dry air (Barbosa Filho et al., 2007). The temperature and humidity index (THI) (equation 2) was proposed by Thom (1958).

$$H = 6.7 + 0.243 * T + \left\{\frac{RH}{100} * \ 10^{\frac{7.5*T}{237.3+T}}\right\} \qquad \text{Eq. (1)}$$

$$THI = T + 0.36 * Tdp + 0.42 \qquad \text{Eq. (2)}$$

Where: H = specific enthalpy (kcal/kg dry air); T = air temperature (°C); RH = relative humidity (%); THI = temperature and humidity index; Tdp = dew point temperature (°C).

2.3.2 Evaluation of the physical properties of bedding and nests

2.3.2.1 Determination the humidity of litter and nests

Litter was collected twice during the experimental period. Samples were collected at three different points in the shed (beginning, middle, and end), with each collection consisting of 20 samples. Each sampling point corresponded to a circle of approximately 20 cm in radius and 7 cm in height, taken with the help of an auger and shovel. The 20 bedding samples collected were then divided into 4 portions, resulting in 5 composite samples, which were decompressed, homogenized and packed in disposable aluminum trays. The same procedure was used to analyze the nest litter.

The samples were then pre-dried in a forced-air oven at 55°C for 72 hours, followed by final drying in an oven at 105°C for 24 hours. After the drying period, the samples were finally weighed and the results calculated according to the equations for water content on a wet basis (Equation 3) and water content on a dry basis (Equation 4):

$$Ucn(\%) = (A - B) * \frac{100}{C} \qquad \text{Eq. (3)}$$

$$DM = 100 - Ucn \qquad \text{Eq. (4)}$$

Where: Ucn (%) = litter and nest moisture; DM = dry mass (%); A= crucible weight + sample weight; B= crucible weight + sample weight after drying (g); C= sample weight (g).

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2.3.2.2 Determination of the pH of the bedding and nests

The determination of pH in litter and nests was performed with 20 g of bedding samples dissolved in distilled water at a ratio of 1:4 (bedding sample:water) (Camargo and Valadares 1980). The mixture was allowed to stand for one hour, and then the pH was read using a digital pH meter.

2.3.2.3 Determination of ammonia released from the bed

In 500 mL flasks, 70 g of the collected litter was placed together with a beaker containing 10 mL of boric acid 2% (m/v) (50 mL) and capped. The flask was incubated at a constant temperature of 30°C for 24 hours. The ammonia fixative solution was titrated with 0.05 N sulfuric acid (Hernandes et al. 2002). The results were expressed in milligrams of ammonia released, calculated using Equation 5:

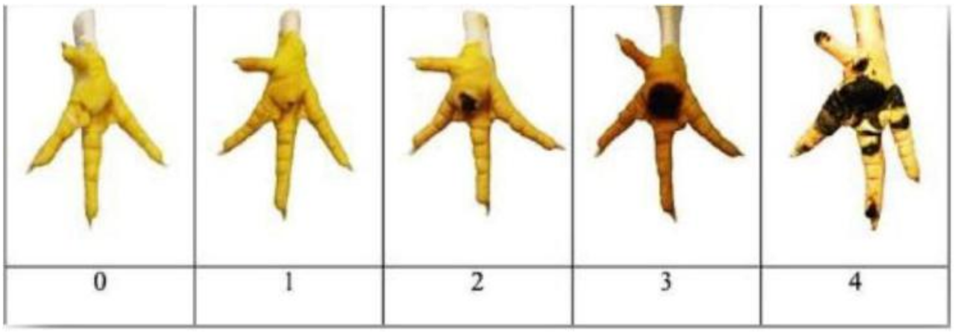
$$A = (Vt * N * 17) \qquad \text{Eq. (5)}$$

Where: A = mg NH3; Vt = the volume of HCl solution used in the titration (mL); N = the normality of the acid used (0.0543N).

2.3.3 Pododermatitis Assessment

Pododermatitis was assessed monthly by observing the integrity of the plantar cushion, based on Welfare Quality (2009). The birds were selected at random, then both feet of each bird were observed, and the severity of the dermatitis was determined by the highest score observed. The scores ranged from 0 for no evidence of foot pad dermatitis to 4 (Figure 2) for a severe lesion.

Figure 2 - Pododermatitis score for birds from the Welfare Quality® protocol Source.



Source: Research data.

2.3.4 Analysis of warping conditions

Birds were randomly selected, and their external appearance assessed. Deformities were recorded for the following body regions: head, neck, breast, tail, wings and cloaca region. The scores used for the scoring were taken from the scores in Table 1:

Table 1 - Warping evaluation scores for each region evaluated.

Scores	Description
4	No damage, feathers intact
3	Slightly broken feathers
2	Very broken feathers, without exposing the skin
1	Featherless area with skin lesions

Source: Research data.

2.3.5 Respiratory Rate and Surface Temperature of Laying Hens

The measurement of this parameter was carried out once a week at the following times: 08:30 AM, 11:30 AM, and 02:30 PM. The animals were randomly selected, totaling 45 birds. Surface temperature data, including crest, dewlap, and shin temperatures, were measured on the same animals using a Digital Infrared Thermometer with laser sight, GM-300 model, temperature range (-50°C to 380°C), and accuracy of ± 1.5 degrees.

2.3.6 Behavioral assessments

Based on preliminary observations at the study site, an ethogram was developed (Table 2).

Table 2 - Behavioral ethogram developed for free-range laying hens.

Behavioral categories	Behaviors	Description
Natural Behavior	Flapping wings	Flapping both wings
	Pecking at objects	Pecking directed at objects, excluding the waterer or feeder
	Scratching the ground	Walking with head down searching for invertebrates and small stones on the ground/bedding. Occasionally, it scrapes the surface with its claws and takes a step or two back to observe any potential prey that was unearthed
	Stretching wings	Wing stretching behavior
	Seeking shade	Walks in the pasture seeking shade under trees or bushes
Investigative Behavior	Investigating feathers	Investigates its own feathers or those of its companion with its beak
Exploratory Behavior	Visiting nests	Visits the nests and vocalizes "coo-coo-coo"
Stress Behavior	Aggressive pecking	Strong pecking at another bird, provoking aggressive or defensive reaction, usually directed at the upper region of the head and crest or at the lower dorsal region of the neck and cloaca
	Fighting	Act of pecking any part of another bird's body aggressively
	Cannibalism	They start pecking at each other's toes, or when an injured bird becomes the target of its companions seeking blood from any wound
Feeding Behavior	Capturing insects	Goes out to forage in the pasture or walks on the bedding/floor, searching for insects to capture and eat
	Eating	Stands in front of the feeder and eats feed
	Drinking	With the body in a neutral position, dips its beak into the water/drinker and quickly lifts its head to swallow
	Foraging	Consuming and/or pecking at the vegetation in the pasture area
Reproductive Behavior	Nesting behavior	Remains seated in the nest in a state of observation around her
	Mounting reflex without a rooster	The bird crouches when other approaches as a mounting reflex
	Posture	Sits in the nest with confirmation of the presence of an egg

Social Behavior	Interacting	Positive interaction of investigating feathers, perching, foraging, pecking at objects, etc
	Chasing	Act of chasing another bird
	Non-aggressive pecking	Pecking lightly at other birds, usually in the lower ventral region of the neck, back, base and tip of the tail, and abdomen
	Vocalizing	Maintains an upright posture and remains alert. The head remains still, and the beak opens to emit the sound "coo-coo-coo"
Locomotion Behavior	Walking	With rhythmic steps, projects the head forward followed by the body
	Running	Runs with or without flapping wings, with the neck extended and the body leaning forward
	Perching	Climbs and remains on the perch
	Flying	On both legs, leans forward to generate momentum and flaps its wings to take flight
Maintenance Behavior	Sand bathing	Rolling in the bedding substrate or on the ground in the pasture area, spreading it over the body
	Sunbathing	Basks in the sun, with wings and tail slightly open. Others may incline during sunbathes, with the wing on the opposite side raised so that the underside is fully exposed
	Scratching the head	Raises one leg with toes slightly apart up to chest height and keeps the head turned to the side, scratches the back of the head, periophthalmic region, neck, or ear
	Stretching legs and wings	From the neutral position, raises the body, extends the neck forward, and stretches the leg and wing to the side facing slightly downward; the body is slightly tilted to the opposite side to maintain balance. The tail is fanned out. It is quite noticeable the effort the bird makes to stretch the muscles of the entire body, including those of the toes
	Cleaning beak	Scrapes, alternately, the sides of the beak against the perch or bedding
	Shaking and fluffing feathers	Fluffs up the feathers on the head, neck, chest, belly, and back and shakes them in short, rapid semicircles
Resting Behavior	Lying down	In a state of rest, with the chest and head supported on the bedding
	Resting	Keeps one leg stretched out, tucks the other, and remains in the neutral position
	Sleeping	In a neutral position with one leg tucked under the feathers of the belly, turns the head towards the back and hides the beak and cere in the ruffled plumage
	Neutral position	Keeps the legs still and slightly apart; the tail is drooped and the wings are close to the body
	Sitting	Body in contact with the floor, ground, or bedding

Source: Research data.

Behavioral observations were conducted weekly in three periods (morning, from 08:30 AM to 09:30 AM, afternoon, from 12:00 PM to 01:00 PM, and late afternoon, from 03:30 PM to 04:30 PM). Before starting data recording, the observer entered the poultry house, waited for approximately 40 minutes for the birds to adapt, and then began the evaluations. It is worth noting that the entire procedure was carried out on days that did not coincide with the physiological variables measurements to avoid interference.

2.3.7 Corticosterone Analysis

The excreta of the animals were collected twice because it showed a large amount of hormones (steroids) associated with stress, such as androgens, estrogens and progestogens; glucocorticoids (corticosteroids), cortisol and dehydroepiandrosterone; and mineralocorticoids.

For sample collection, durable plastics were distributed at strategic points in the poultry house, where they awaited the bird's excretion. Samples were then swiftly collected, placed in Eppendorf tubes, and stored on ice. Subsequently, they were kept in a -90°C freezer until analysis. Each fecal sample was thawed beforehand, and then 0.5 g aliquots were weighed on an analytical balance and added to 5.0 mL of 90% ethanol. The samples were then agitated on a vortex apparatus for 15 minutes, centrifuged at 3,000 rpm for 10 minutes, and the supernatant was transferred to 1.5 mL polypropylene microtubes. Fecal corticosterone metabolites were quantified using the double antibody radioimmunoassay (I125 RIA) technique through a commercial diagnostic kit - 11-Desoxycortisol®-RIA-CT.

2.4 Statistical Analysis

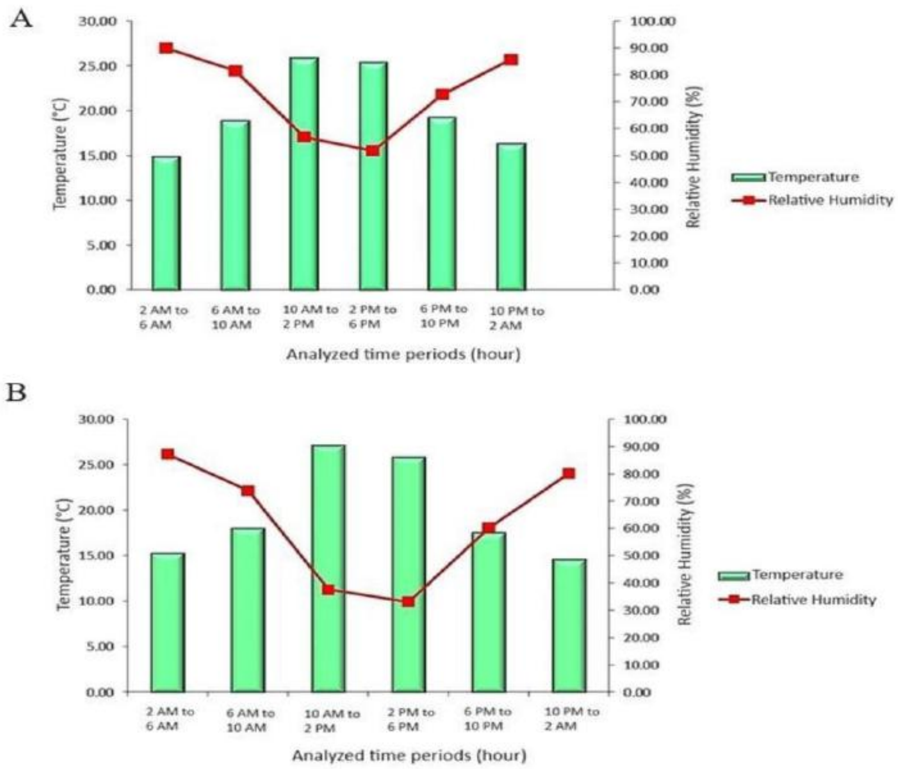
The temperatures of the crest, dewlap, and shin, respiratory rate, and frequency of behaviors in the internal and external areas of the poultry house were analyzed as a function of the independent variables: bird age (in weeks) and time (08:30 AM to 09:30 AM, 12:00 PM to 01:00 PM, and 03:30 PM to 04:30 PM). Temperature data were analyzed using two-way analysis of variance (ANOVA) and, when significant, compared using the Tukey test. Respiratory rate was analyzed using generalized linear models (GLM) assuming a Poisson distribution, and when significant, comparisons were made using the Wald test. Behavior data were also analyzed using generalized linear models (GLM) assuming a Poisson distribution, and in case of overdispersion, the Quasi-Poisson distribution was used. Model adequacy was assessed through half-normal probability plots with envelope simulation, and significant comparisons were made using the Wald test. Corticosterone levels were analyzed through one-way analysis of variance (ANOVA), using logarithmic transformation of corticosterone data, and when significant, means were compared using the Tukey test. Model adequacy was assessed through half-normal probability plots with envelope simulation. Statistical analyses of the studied variables were performed using the R software.

3. Results

3.1 Climatic Parameters

Figure 3 represents the variations in temperature and relative humidity in both the indoor and outdoor environments of the shed. It was found that the temperatures throughout the entire experimental period ranged from 14.89°C to 25.89°C indoors and from 14.63°C to 27.10°C outdoors. The relative humidity indoors corresponded to 73.05%, while it was 62.01% outdoors.

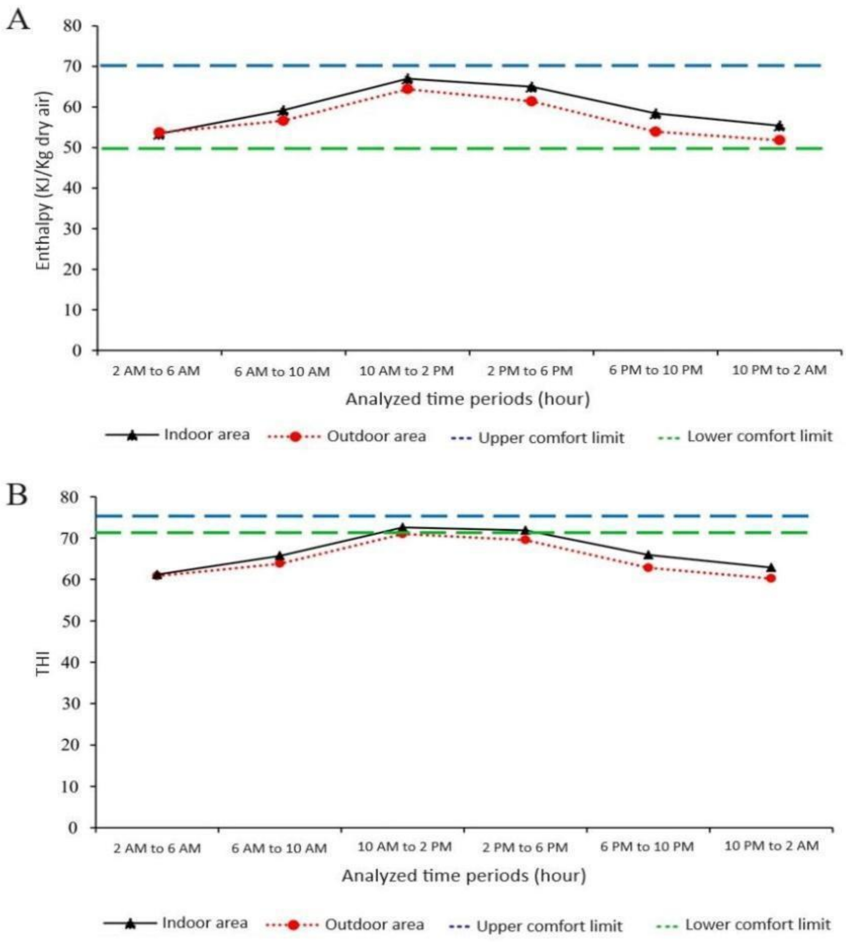
Figure 3 - Variation of temperature and relative humidity in the internal (A) and external (B) microenvironments of the barn during the experimental period.



Source: Research data.

The Enthalpy Index (Figure 4A) showed average values ranging from 53.4 to 67 KJ/kg of dry air in the indoor area of the facility and from 53.7 to 64.4 KJ/kg of dry air in the outdoor area. Regarding the average values of THI (Figure 4B), they ranged from 60.19 to 72.54.

Figure 4 - Values of enthalpy (A) and THI (B) in the internal and external areas of the house and the values considered ideal for free-range laying hens with their respective upper and lower comfort limits.



Source: Research data.

3.2 Air quality and physical properties of the bedding and nests

The moisture content, pH, and concentration of ammonia (NH3) in the bedding and nests are represented in Table 3. The concentration of ammonia in the bedding was 5.49 ppm. The moisture content of the bedding and nests ranged from 17.62 to 23.23%, and the pH ranged from 7.50 to 8.15.

Table 3 - Moisture content (%), pH, and volatile ammonia from the bedding and nests of free-range laying hens.

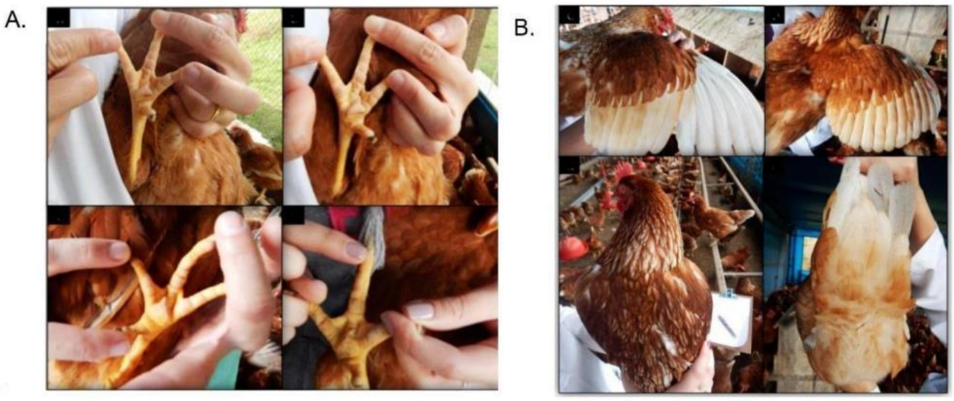
	Features		
Location Analyzed	Moisture content (%)	pH	Volatile ammonia (ppm)
Aviary bedding	23.23	8.15	5.49
Lower nests	17.62	7.70	-
Upper nests	18.51	7.50	-

Source: Research data.

3.3 Evaluation of pododermatitis and feathering conditions

There was no incidence of pododermatitis in the birds, showing a score of 0 (absence of lesions) throughout the experimental period (Figure 5A). Additionally, it was found that the feathering conditions of the animals scored 4 (Figure 5B), indicating no damage, with intact feathers in both evaluated regions.

Figure 5 - Plantar pad (A) and feathering conditions (B) of free-range laying hens.



Source: Research data.

3.4 Surface temperature and respiratory rate of laying hens

The Table 4 presents the mean values of temperatures referring to the comb, wattles, and shanks of the birds measured weekly. There was a significant interaction between the observation period and the age of the birds on the comb temperature (F9,493=3.059, P=0.00141), wattle temperature (F9,493=5.901, P<0.001), and shank temperature (F9,493=3.031, P=0.00154). Additionally, it was observed that the effect of bird age was not significant on the variation of respiratory rate; thus, the mean respiratory rate was 19.82 breaths/min.

Table 4 - Mean temperatures of the comb, wattles, and shanks (°C) of free-range laying hens.

Age of the birds (weeks)	Comb Temperature (°C)		
	08:30 AM	11:30 AM	02:30 PM
18	32.12 (±2.31) Ba	31.63 (±1.74) Aa	33.84 (±1.77) Aa
19	31.47 (±1.51) Ba	33.18 (±1.72) Aab	34.06 (±1.55) Ab
20	33.16 (±2.76) Ba	43.29 (±1.79) Bb	33.93 (±1.76) Aa
21	29.54 (±2.71) Aa	30.83 (±2.62) Aab	32.71 (±1.86) Ab
22	33.20 (±1.63) Ba	34.32 (±1.75) Aa	34.94 (±1.50) Aa
23	30.73 (±1.45) ABa	31.13 (±1.60) Aa	31.99 (±1.51) Aa
Age of the birds (weeks)	Wattle Temperature (°C)		
	08:30 AM	11:30 AM	02:30 PM
18	27.21 (± 2.21) Ba	28.29 (±1.49) Ba	30.31 (±1.38) Ba
19	25.40 (± 2.96) Ba	27.80 (±2.12) Ba	29.23 (±1.4) Ba
20	27.90 (±2.59) Ba	29.37 (±1.97) Ba	29.06 (±2.27) ABa
21	20.88 (±2.17) Aa	22.98 (±2.15) Aab	25.34 (±1.81) Ab
22	26.50 (±2.61) Ba	31.00 (±1.80) Bb	30.72 (±2.66) Bab
23	21.42 (±1.42) Aa	22.54 (±1.74) Aab	25.28 (± 1.61) Ab
Age of the birds (weeks)	Shank Temperature (°C)		
	08:30 AM	11:30 AM	02:30 PM

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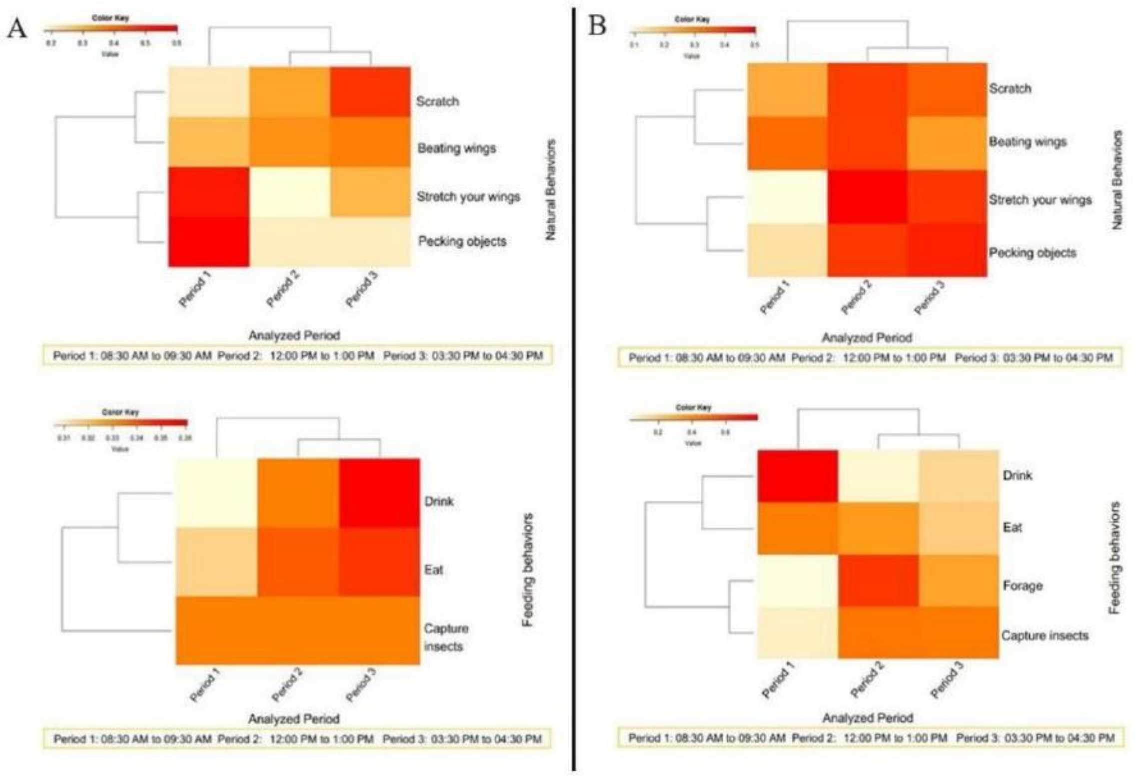
18	28.12 (±3.04) ABCa	29.87 (±1.89) Ca	31.11 (±1.94) BCa
19	28.31 (±3.06) BCa	29.65 (±2.43) Ca	31.81 (±2.62) BCa
20	29.28 (±3.07) Ca	30.72 (±3.68) BCa	30.40 (±3.75) BCa
21	21.90 (±3.26) Aa	24.32 (±2.80) ABa	26.70 (±2.68) ABa
22	28.23 (±3.64) ABCa	32.24 (±2.93) Ca	32.34 (±2.86) Ca
23	23.04 (±2.37) ABa	23.60 (±2.52) Aa	25.18 (±1.27) Aa

Different uppercase letters indicate significant differences (P<0.05, Tukey test) in the comparison between rows (weeks), and different lowercase letters indicate significant differences (P<0.05, Tukey test) in the comparison between columns (time). Source: Research data.

3.5 Behavioral expressions

The proportions of natural and feeding behaviors performed by laying hens in the indoor and outdoor areas of the shed are demonstrated in Figure 6, using the heatmap graphical tool, which is useful for representing data that naturally aligns with numerical data in a two-dimensional grid, where the value of each cell in the grid is represented by a color. It was observed that the frequency of scratching behavior (37%), wing flapping (46%), drinking (53%), and eating (58%) were most frequent in period 3. On the other hand, pecking at objects (3%), stretching wings (9%), and capturing insects (2%) stood out in period 1. Additionally, in period 2, these behaviors also stood out, except for stretching wings. Furthermore, the behaviors recorded in the outdoor area of the shed showed that the behavioral frequency of scratching (47%), stretching wings (6%), wing flapping (30%), foraging (44%), and capturing insects (48%) prevailed in period 1. Pecking at objects predominated in period 2 (13%), and the activities of drinking and eating were similar to those in the indoor area of the shed.

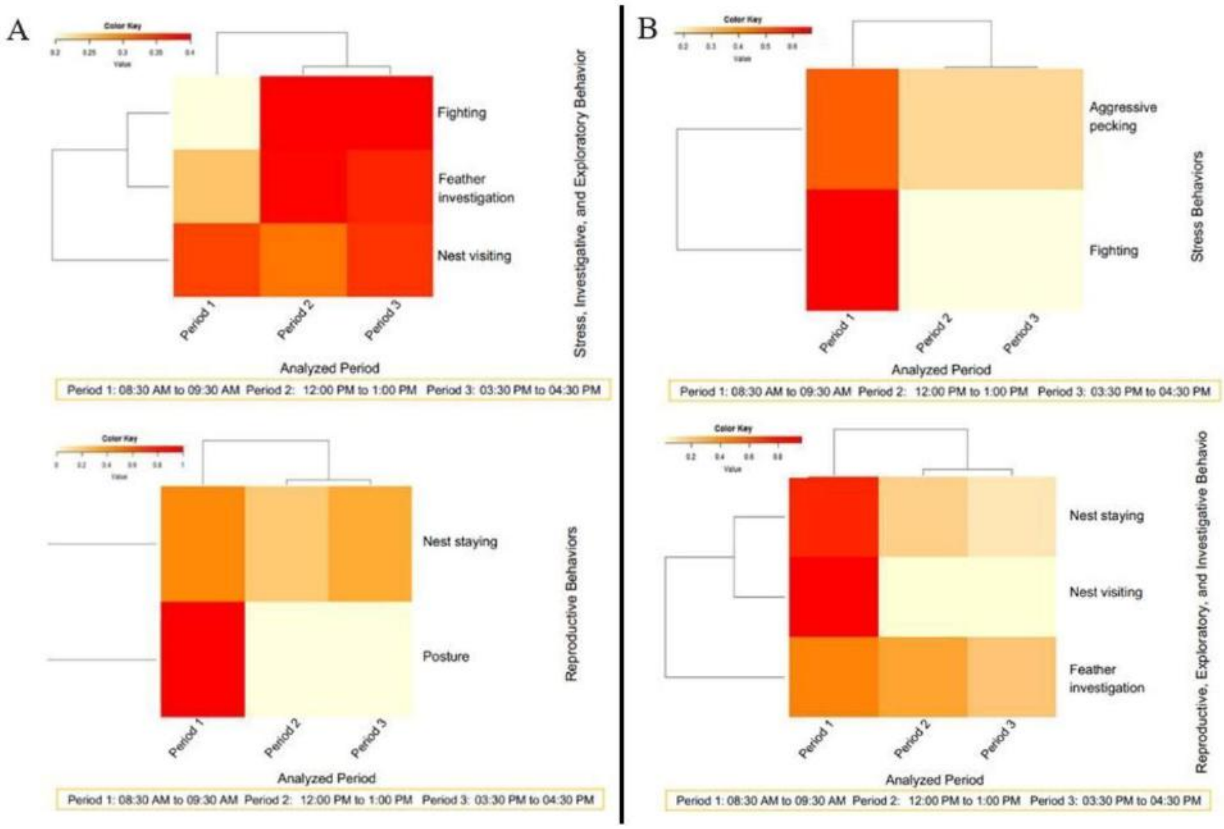
Figure 6 - Frequency pattern of natural and feeding behavior observed in laying hens in the indoor area (A) and outdoor area (B) of the shed.



Source: Research data.

The behavioral categories of stress, investigative, exploratory, and reproductive are presented in Figure 7. It was observed that the behavioral expressions of fighting (2%) and feather investigation (52%) stood out in period 2 in the indoor area of the shed. However, the same behaviors in the outdoor area were more pronounced in period 3, as well as the frequency of nest sitting (9%) (or brooding behavior) and aggressive pecking (2%). Additionally, the reproductive behaviors in the indoor area of the shed were frequent in period 1 and subsequently in period 3. On the other hand, the behavior of visiting nests (50%) was prominent in period 3 in both observed areas.

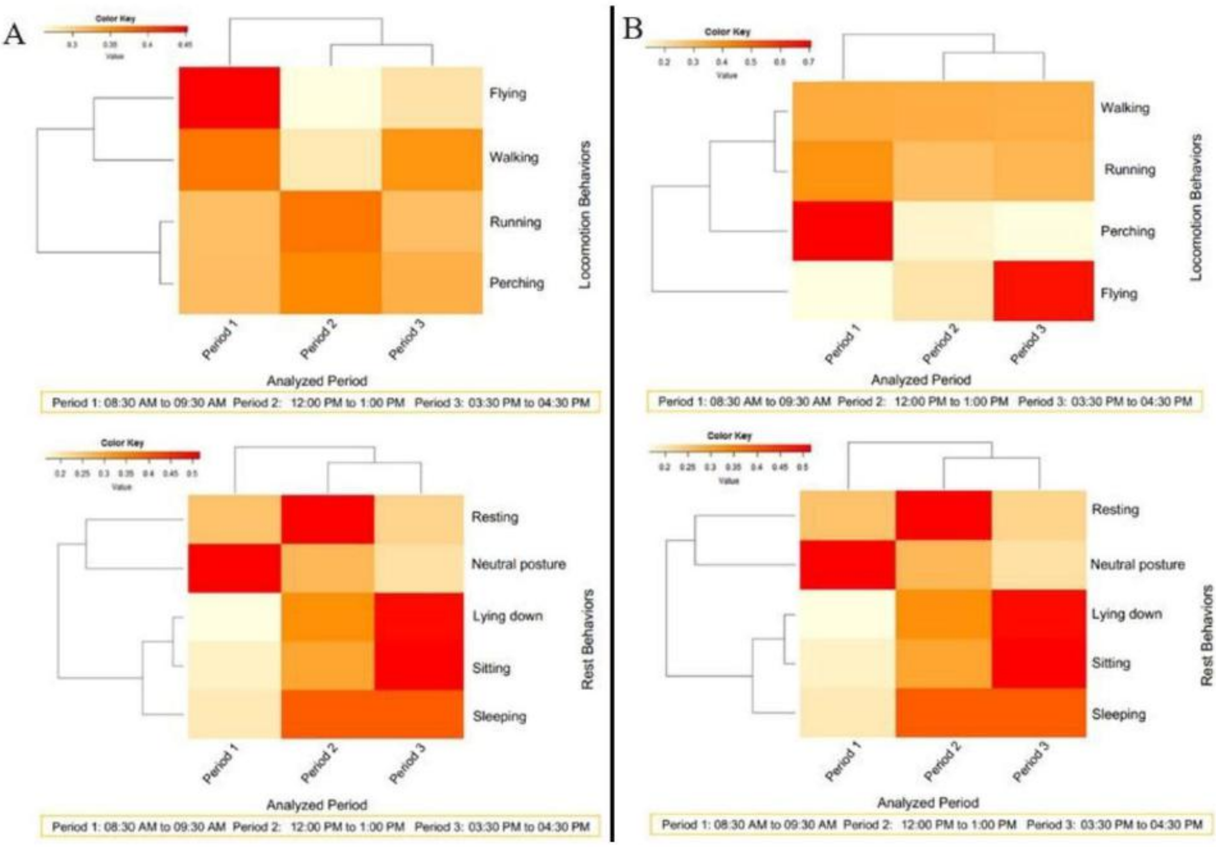
Figure 7 - Frequency pattern of stress, investigative, exploratory, and reproductive behavior observed in laying hens in the indoor area (A) and outdoor area (B) of the shed**.**



Source: Research data.

Observing Figure 8, it can be seen that the behavioral frequency of walking (65%) was equal in both areas, prevailing in period 1. On the other hand, the behavior of flying stood out in period 1 with a frequency of 19% (indoor area of the shed) and in period 2 (21%) in the outdoor area of the shed. The behavioral expressions of running and perching showed a periodicity of 26% and 34% in period 2 (indoor area) and in period 3 (outdoor area) of 37% and 31%, respectively. The behavioral expressions of lying down and sitting stood out in period 3 with a frequency of 3% and 22%, respectively, in both areas, as well as the behavior of resting (20%) and sleeping (2%) in periods 1 and 3. Additionally, the neutral position behavior stood out in period 2 with a frequency of 34%.

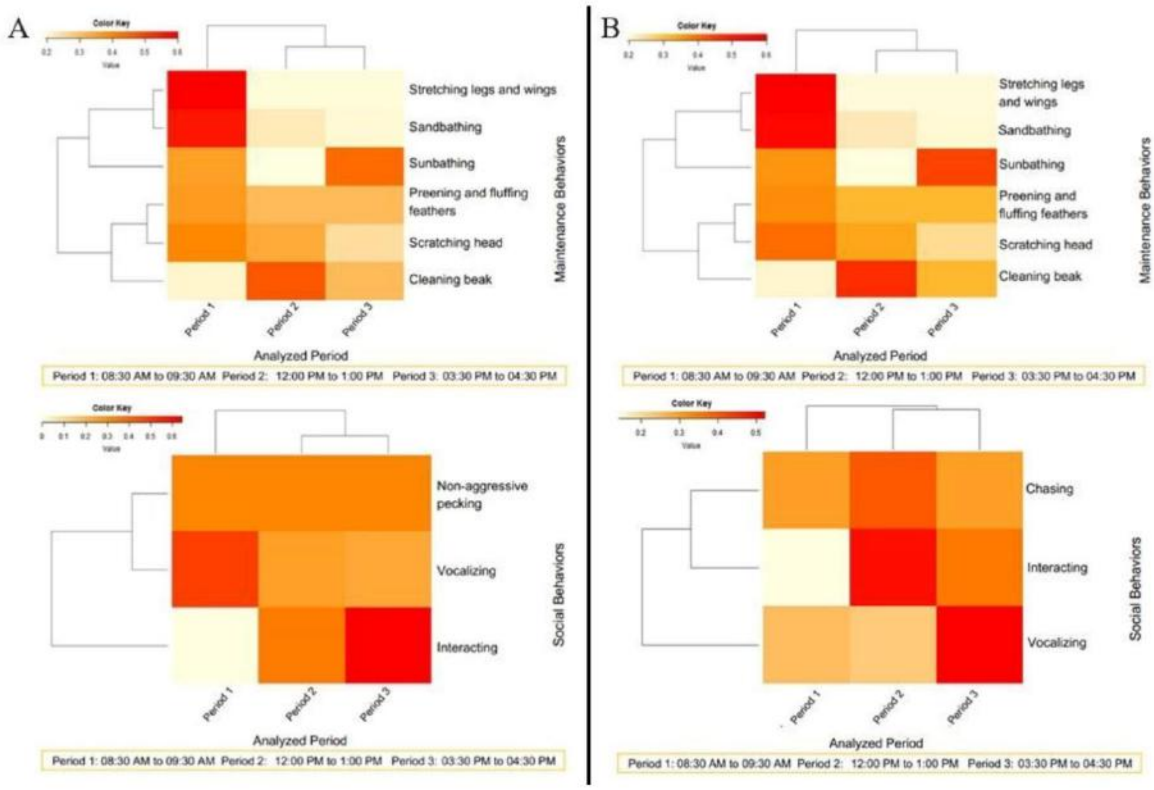
Figure 8 - Frequency pattern of locomotion and resting behavior observed in laying hens in the indoor area (A) and outdoor area (B) of the shed.



Source: Research data.

The frequency of maintenance and social behavioral categories are shown in Figure 9. It was observed that movements such as stretching legs and wings, dust bathing, shaking and fluffing feathers, and head scratching were evident in period 3 in both areas, with frequencies of 27%, 22%, 9%, and 35% respectively. Similarly, sunbathing behavior expressed a frequency of 16% in period 2, and beak cleaning (10%) in period 1 in the analyzed areas. The frequency of behavioral expressions related to non-aggressive pecking and chasing ranged from 1% to 4%, standing out in period 1. Interaction behavior (9%) was higher in period 3, and vocalization (43%) in period 1 (indoor area of the shed), and they stood out in periods 1 and 3 in the outdoor area, indicating frequencies of 7% and 25%.

Figure 9 - Frequency pattern of maintenance and social behavior observed in laying hens in the indoor area (A) and outdoor area (B) of the shed.

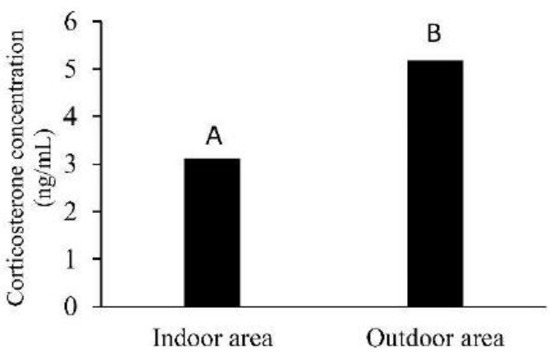


Source: Research data.

3.6 Quantification of corticosterone levels

Stress is one of the main parameters for assessing animal welfare, and corticosterone has been used as a biological indicator of stress in many species. It was observed in Figure 10 that there was a significant difference ($F_{31,2}$=11.87, P<0.001) in the quantification of corticosterone in the analyzed areas, with mean values of 3.11 and 5.19 ng/ml for the indoor and outdoor areas, respectively.

Figure 10 - Quantification of corticosterone levels in the excreta of free-range laying hens.



Source: Research data.

4. Discussion

The climatic parameters indicate that the animals were within the corresponding thermoneutral range for adult laying hens, where the animals find suitable conditions to express their best productive and behavioral characteristics. It is worth noting that the increase in environmental temperature results in a decrease in feed intake, consequently reducing the intake of essential energy nutrients and decreasing egg production (Samara et al. 1996).

Regarding relative air humidity, the environment is considered comfortable for adult birds when it presents values between 50 to 75% (Tinôco 2001). In high humidity values, birds become more sensitive to heat, which greatly influences animal welfare and productivity. Therefore, the obtained values remained within the expected range for laying hens in the production phase.

The environment considered comfortable for laying hens should have an THI ranging between 71 and 75 (Gates et al. 1995). Values above 75 to 79 represent situations of alert and danger for production, and between 79 and 84 indicate emergencies, requiring urgent measures to avoid loss of the flock. Thermal comfort inside poultry facilities is important because inadequate conditions negatively affect animal performance (Welker et al. 2008). Therefore, since the thermal comfort indices (H and THI) obtained inside and outside the shed remained within the comfort zone for the birds, it can be considered that suitable and non-stressful situations for laying hens were characterized.

The characteristics of the environment in the free-range system resulted in good quality bedding, nests, and volatile ammonia concentration. Regarding the concentration of ammonia in the bedding, the value was lower than recommended in the literature, with a tolerance limit of 20 ppm (Manning et al. 2007). The concentration levels of NH_3 can be influenced by factors such as humidity, bedding usage time, management, pH, and stocking density, which can accelerate the microbial decomposition of uric acid from excreta, increasing NH_3 production. A high concentration of ammonia causes problems such as reduced animal weight, eye injuries, and underdevelopment of the carcass (Nääs et al. 2007; Vitorasso and Pereira 2009; Furtado et al. 2010).

Another extremely important factor is air quality in poultry farming, as inadequate ventilation increases gas concentrations inside the facilities, decreasing oxygen concentration and creating an environment conducive to respiratory tract diseases. Therefore, ventilation control is important to keep humidity and ammonia below critical limits (Menegali et al. 2012).

Bedding humidity is one of the major concerns in poultry production due to its negative impact on the health, welfare, and productive performance of birds. However, it was found that the values obtained were below 35%, considered within ideal parameters (Cobb-Vantress 2008). Regarding bedding pH, the accumulation of ammonia and fecal material causes it to increase, typically ranging from 7 to 8.5. Therefore, the pH levels of the bedding and nests are within the desirable limit.

The absence of pododermatitis is related to the quality of bedding, air, and environment provided by the free-range system because its presence is attributed to inadequate bedding conditions, particularly due to climatic and ventilation factors (Shepherd and Fairchild 2010), excess moisture, and high density. Additionally, Rodriguez-Aurrekoetxea and Estevez (2016) observed a lower incidence of pododermatitis and plumage damage in birds in the free-range system.

The quality of the environment in the system also resulted in satisfactory feather conditions. Feathering in birds involves complex physiological mechanisms influenced by environmental, density, nutritional, hormonal, and genetic factors, as well as their interaction (Krueger 1994).

The conditions to which the animals were subjected contributed to thermal comfort variables. Regarding the temperatures of the comb, wattle, and shank, the values are within the range recommended by the literature regardless of the time evaluated. The ideal temperature for the comb for laying hens corresponds to 34.5°C to 35.9°C, wattle 34.5°C to 35°C, and shank 37°C to 37.5°C (Shevel'Ko 1967; Hester 2005).

It is noteworthy, as discussed earlier, that the ambient temperature, as well as the relative air humidity, corroborated with the results of surface temperature and respiratory frequency. During the experimental period, the maximum recorded temperature was 25.89°C and the minimum was 14.89°C, while the relative air humidity ranged from 51.72% (minimum) to 89.9% (maximum). It is known that birds maintain a constant body temperature when the ambient temperature is within the thermoneutral range. Dahlke et al. (2005) calculated that the total surface area of the comb, wattles, and shanks of the birds corresponds to 16% of the total body surface area, reflecting the importance of these body regions in heat dissipation by the birds.

Nääs et al. (2010) found that the temperature of the comb, wattles, and shanks in broiler chickens in the morning ranged from 39.0°C to 39.5°C; 39.5°C, and in the afternoon from 40.0°C to 39.5°C; 41.0°C, respectively. On the other hand, in studies conducted with laying hens, found that the shank temperature ranged from 36.2°C to 38.7°C in the free-range system (Camerini et al. 2013).

With laying hens kept in cages at different population densities, the authors obtained mean comb temperatures in the morning of 33.96°C and in the afternoon of 35.35°C (Garcia et al. 2015). Regarding wattle temperature, they found values of 27.79°C (morning) and 30.36°C (afternoon), and for shank temperature, 34.3°C (morning) and 35.4°C (afternoon). Therefore, the results of comb and wattle temperatures found corroborate with these studies.

Additionally, the average respiratory rate of 19.82 movements per minute strengthens the comfort of the birds in the free-range system. In laying hens, the respiratory rate can vary from 23 movements per minute in a thermal comfort environment to 273 movements per minute in situations of high temperatures (Kassim and Sykes 1982). Studies conducted by Amaral et al. (2011), with broiler chickens kept in commercial sheds, showed average respiratory rate values of 68 mov./min. Castilho et al. (2015) found that laying hens kept at different densities in cages had respiratory rate values between 28 and 36 mov/min during various periods of the day.

Therefore, the averages of respiratory rate and surface temperature demonstrated that in thermoneutral situations, birds do not need to intensively utilize mechanisms such as panting to maintain constant body temperature, indicating that the birds kept their body temperature within ideal physiological standards.

Taking into consideration all these aspects of environment and animal welfare, it is possible to observe the characteristic behaviors of birds in the free-range production system. When birds are under stress, they alter their behavior to help maintain body temperature within normal limits (Pereira et al. 2007). There is a decrease in feed intake and increased water intake when birds are in environments above the thermoneutral zone, in addition to an increase in pecking frequency (Silva et al. 2006; Barbosa Filho et al. 2007).

The free-range system provided an enriching environment, offering birds other options to spend time on different activities. Abd El-Hack et al. (2017) reported that birds tend to feed more at the beginning or end of the day, or both, but not at noon. Additionally, they stated that visual cues act as synchronizers on feeding behavior, explaining why animals tend to feed together.

Another point is the water consumption in the conventional system, as birds spend most of the day drinking water, which can be explained by the lack of environmental enrichment. The increase or decrease in this behavior may be related to the stress conditions to which the animal may be subjected, mainly due to confinement and the inability to perform natural behaviors.

The act of pecking was significant and is characteristic and natural for birds, identified when the bird explores its territory with its feet and beak. Haas et al. (2010) found that the prevention or reduction of pecking behavior significantly aggravates bird stress, where there seems to be a natural substitution of this pecking behavior for feather pecking, varying in intensity according to the lineage and age of the flock. Hens prefer environments where they are offered opportunities to peck and clean feathers (Nicol et al. 2009). Additionally, Schwean-Lardner et al. (2010) concluded that behaviors indicating physical activity can be used to assess the health and comfort or welfare status.

The behavior of foraging and capturing insects by the birds was significant, involving activities outside the production module, such as pecking at vegetation in search of food, as well as preying on insects. Grazing contributes to the reduction of problems such as cannibalism (Dawkins 1989).

The behavior of pecking at objects stood out in the external area of the shed. The reason that may have influenced the high incidence of this behavior was the possibility for the birds to explore their environment where they are being raised. Chickens kept in cages show a tendency to peck at metal screens or any other object (feeder), a behavior that may reflect a lack or frustration of access to substrate, as well as exploration of the environment, foraging, or sand bathing (Mollenhorst et al. 2005).

Regarding aggressive pecking, which occurred quickly, it had a frequency of only 2% and showed little expression of this behavior. Unlike birds raised in cages, birds exhibit aggressive behavior, probably as an indication of the greater stress burden suffered by birds in confinement conditions (Barbosa Filho et al. 2007).

For the behavior of fighting, Kolb (1984) comments that hierarchical fights (strong pecks on the crest, head, and neck of the opponent) occur from the early stage as a playful behavior. Regarding the behavior of birds investigating their own feathers or those of other birds with their beaks, this action can be considered indicative of well-being because this behavior is characteristic and natural for birds.

The act of perching is a necessary behavior for birds, with benefits related to strengthening the leg bones through physical exercise, which can reduce the incidence of fractures as they age. Birds exposed to areas with free movement positively affect health and well-being (Bessei et al. 2006). The behaviors of resting and sleeping are considered important ethological needs for birds (Pickel et al. 2011) as they prevent potential physiological changes in the body, such as diseases or stress responses.

The sand bathing activity has both behavioral and physical effects as it regulates the lipid layer of the feathers and keeps them in good condition, besides being a way for birds to dissipate heat to the environment. The time of day has a significant influence on the expression of bird behaviors due to biological rhythms, especially the photoperiod. In this sense, the birds showed a preference for performing this activity in the afternoon.

The act of scratching the head is crucial for birds, as a higher intensity, duration, and frequency of this behavior indicate better well-being (Bracke and Hopster 2006). Sunbathing behavior is related to the need to eliminate parasites, maintain feather health, and synthesize vitamin D3 for healthy bone development. On the other hand, the shaking and fluffing of feathers remove any dirt or dust from the birds' bodies, help the feathers return to their normal position, and in some cases, relieve tension (Koelkebeck et al. 1987).

The cleaning of the beak can be explained by two reasons Choudary et al. (1972): to show dominance of personal territory in the presence of another bird, or when alone, it indicates something bothering its beak and it seeks to clean it on the ground or in the bedding. Another behavior is body stretching, to relieve the tension of spending long periods on its feet.

During the observations, the system provided few opportunities for non-aggressive pecking expression, as the birds had physical space to explore with free access to the paddock. On the other hand, the birds vocalized more frequently. Low-frequency vocalizations (clucking) are used in maintaining social contact, while high-frequency vocalizations (songs, screams, etc.) are more related to states of excitement (Spinka 2012).

Social behaviors such as chasing and interacting refer to the interaction between individuals, which can be cooperative (mating, friendly interaction) or conflicting (fighting, territory dispute). This behavior can be influenced by various external and internal factors. An environment that is more abundant in food and territory allows for a more amicable relationship between individuals and a well-defined hierarchy among animals. Additionally, there was no incidence of cannibalism among the animals.

In light of the above, it can be inferred that the free-range system provided autonomy to the hens, allowing them to choose the most appropriate location to stay between the outdoor and indoor areas of the barn, as well as to express their natural behaviors such as simple acts of dust bathing, sunbathing, foraging, among others.

Therefore, this system was favorable for the welfare of laying hens, especially in the outdoor area of the barn, as it demonstrated a greater richness of behaviors, particularly natural behavior patterns. It is worth noting that stress can have significant impacts on behavior, immune functions, and reproduction in animals (Alm et al. 2016).

As a way to assess the welfare of the hens, obtaining corticosterone levels allowed us to verify that they were at appropriate levels. Fraisse and Cockrem (2006) observed plasma and fecal corticosterone levels at values of 12.45 and 12.31 ng/g for fecal samples, and 12.00 and 8.5 ng/ml in plasma. Dawkins et al. (2004) investigated two groups of laying hens, monitoring corticosterone levels in excreta over five days, finding corticosterone concentrations ranging from 21 to 30 ng/g.

The values found were 3.11 ng/ml in the indoor area of the shed and 5.19 ng/ml in the outdoor area. Gibson et al. (1986) observed that the quantification of corticosterone in the plasma of free-range laying hens corresponded to 4.5 ng/mL. It is important to mention that the basal concentration of corticosterone in laying hens is estimated to be between 10 and 30 ng/mL (Holmes 1978). It is noteworthy that a possible explanation for the increase in corticosterone levels in the birds in the outdoor area of the shed may be due to alertness to potential predators. However, despite the difference found, the values of the levels are within the appropriate range.

5. Conclusion

Given the above, we can report that the birds exhibited a good quality of life in terms of animal welfare, as the environmental conditions and adaptability positively influenced the behavior of the birds in the free-range system. The characteristics of the free-range system environment resulted in the quality of bedding, nests, and the concentration of volatile ammonia, which led to the absence of pododermatitis and the quality of feathering in the birds. Additionally, it showed satisfactory results regarding animal welfare conditions, combined with comfort and thermal stress conditions, with variables such as respiratory rate and surface temperature remaining at satisfactory levels.

The behavioral response for a bird to alleviate a stressful situation can involve exploring the environment; if this is not sufficient, it may exhibit other types of behavior, ranging from vocalization to the expression of natural behaviors. Additionally, corticosterone levels were lower when compared to hens kept in conventional systems. Therefore, behavioral, physiological, health-related, and production-related assessments, when combined, constitute effective tools in assessing animal welfare.

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