REVISTA CIENTÍFICA - RECIMA21 ISSN 2675-6218

BEHAVIORAL RESPONSES OF LAYING HENS UNDER POSITIVE AND NEGATIVE PRESSURE VENTILATION SYSTEMS

COMPORTAMENTO DE GALINHAS POEDEIRAS EM SISTEMAS DE VENTILAÇÃO POR PRESSÃO POSITIVA E NEGATIVA

COMPORTAMIENTO DE GALLINAS PONEDORAS EN SISTEMAS DE VENTILACIÓN POR PRESIÓN POSITIVA Y NEGATIVA

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e737316
https://doi.org/10.47820/recima21.v7i3.7316

PUBLISHED: 03/2026

ABSTRACT

The study compared the behavior of Bovans White laying hens in positive pressure (PPB) and negative pressure (NPB) ventilation systems, analyzing the thermal impacts on hen welfare. Twenty-four 50-week-old hens were observed over three days during morning and afternoon sessions. The experimental design was exploratory and descriptive, with analysis based on relative frequencies and without the application of inferential statistics. Behaviors were classified into welfare categories (feather exploration, idleness) and heat stress indicators (open beak, open wings). In the PPB, with temperatures ranging from 19.2°C to 36.1°C, stress behaviors such as open beak (12%) and open wings (2%) were more frequent in the afternoon, when the temperature reached 36.1°C. Water intake increased due to heat, while comfort behaviors such as lying down (10%) decreased. In the NPB, with more stable temperatures (21.5°C to 26.5°C), behaviors such as lying down (20%) and idleness (12%) were more common, indicating greater thermal comfort. Feeding was the predominant behavior in both systems, with higher frequency in the NPB during

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ISSN: 2675-6218 - RECIMA21

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the afternoon (54%). The results highlight the importance of thermal control in poultry management, indicating that the negative pressure ventilation system, equipped with evaporative pads and exhaust fans, promoted greater thermal comfort and expression of natural behaviors.

KEYWORDS: Animal behavior. Welfare. Thermal stress. Gallus gallus. Poultry production.

RESUMO
O estudo comparou o comportamento de poedeiras Bovans White em sistemas de ventilação por pressão positiva (GPP) e negativa (GPN), analisando os impactos térmicos sobre o bem-estar das aves. Utilizaram-se 24 aves, com 50 semanas de idade, observadas durante três dias, em turnos de manhã e tarde. O delineamento experimental foi exploratório e descritivo, com análise baseada em frequências relativas, sem aplicação de estatística inferencial. Os comportamentos foram classificados em categorias de bem-estar (exploração de penas, ócio) e estresse térmico (bico aberto, asas abertas). No GPP, com temperaturas entre 19.2°C e 36.1°C, comportamentos de estresse, como bico aberto (12%) e asas abertas (2%), foram mais frequentes à tarde, quando a temperatura atingiu 36,1°C. O consumo de água aumentou devido ao calor, e comportamentos de conforto, como deitar (10%), diminuíram. No GPN, com temperaturas mais estáveis (21.5°C a 26.5°C), comportamentos como deitar (20%) e ócio (12%) foram mais comuns, indicando maior conforto térmico. A alimentação foi o comportamento predominante em ambos os sistemas, com maior frequência no GPN à tarde (54%). Os resultados destacam a importância do controle térmico no manejo avícola, indicando que o sistema de ventilação por pressão negativa, com placas evaporativas e exaustores, favoreceu maior conforto térmico e expressão de comportamentos naturais.

PALAVRAS-CHAVE: Comportamento animal. Bem-estar. Estresse térmico. *Gallus gallus*. Produção avícola.

RESUMEN
El estudio comparó el comportamiento de gallinas ponedoras Bovans White en sistemas de ventilación por presión positiva (GPP) y negativa (GPN), analizando los impactos térmicos sobre el bienestar de las aves. Se utilizaron 24 aves, con 50 semanas de edad, observadas durante tres días en turnos de mañana y tarde. El diseño experimental fue exploratorio y descriptivo, con análisis basado en frecuencias relativas, sin aplicación de estadística inferencial. Los comportamientos fueron clasificados en categorías de bienestar (exploración de plumas, inactividad) y estrés térmico (pico abierto, alas abiertas). En el GPP, con temperaturas entre 19.2°C y 36.1°C, los comportamientos de estrés, como pico abierto (12%) y alas abiertas (2%), fueron más frecuentes por la tarde, cuando la temperatura alcanzó 36.1°C. El consumo de agua aumentó debido al calor, y los comportamientos de confort, como acostarse (10%), disminuyeron. En el GPN, con temperaturas más estables (21.5°C a 26.5°C), comportamientos como acostarse (20%) y inactividad (12%) fueron más comunes, indicando mayor confort térmico. La alimentación fue el comportamiento predominante en ambos sistemas, con mayor frecuencia en el GPN por la tarde (54%). Los resultados destacan la importancia del control térmico en la gestión avícola, indicando que el sistema de ventilación por presión negativa, con paneles evaporativos y extractores, favoreció mayor confort térmico y la expresión de comportamientos naturales.

PALABRAS CLAVE: Comportamiento animal. Bienestar. Estrés térmico. Gallus gallus. Producción avícola.

INTRODUCTION

Brazil is recognized as a world leader in chicken meat exports and is the third largest producer and consumer of chicken meat. In addition, it stands out in terms of egg production,

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ranking as the fifth largest egg producer in the world, with a production of 57.7 billion units and exports of 18.5 thousand tons in 2024 (ABPA, 2025). Despite the challenges facing intensive commercial production, ensuring conditions that promote laying hens welfare is essential for maintaining market competitiveness and meeting the growing demands for sustainable and high-quality products (Silva *et al.,* 2021).

Consequently, in recent years, much poultry research has focused on meeting consumer demand for sustainable production that respects the environment and provides good living conditions for animals (Valentim *et al.,* 2019).

The relationship between animal welfare and productivity is complex and multifaceted. To provide good health conditions for hens, it is essential that production is based on the five freedoms proposed by the Farm Animal Welfare Council: freedom from hunger and thirst; freedom from discomfort; freedom from pain, injury, and disease; freedom to express natural behaviors of the species; and freedom from fear and distress (FAWC, 1992). Zootechnical indicators can be used to assess the impact of different management practices on animal welfare and the efficiency of production systems (Mendez *et al.,* 2008; Cadernos técnicos de veterinária e zootecnia, 2012).

Although the five freedoms are fundamental for the welfare of hens, it is common to find production systems that do not fully meet them, such as the spatial restriction imposed by cages in conventional egg production systems, as highlighted by Aguiar *et al.,* (2021), and temperature control, which negatively impacts the quality of life of hens by affecting their natural behaviors and health (Baêta; Souza, 2010b).

1. LITERATURE REVIEW

1.1. Animal welfare and behavior in laying hens

The animal welfare of laying hens is directly related to their ability to express natural behaviors and adapt to their rearing environment. Systems that restrict essential behaviors, such as nesting, exploring the environment, perching, and dust bathing, can compromise the physical and psychological health of hens (Nicol, 1987; Duncan, 1992; Baxter, 1994; Jeon *et al.,* 2025).

Behavior is widely recognized as a reliable indicator of animal welfare, as it directly reflects the interaction between an animal and its environment. Recent studies have shown that hens housed in cage-free systems display greater behavioral diversity and more frequent expression of behaviors associated with positive emotional states, whereas those raised in conventional cages more often exhibit responses related to fear and apathy (Shimmura *et al.,* 2024; Jeon *et al.,* 2025). Furthermore, behavioral responses are correlated with physiological indicators of stress, such as corticosterone levels, reinforcing the use of behavior as a central tool for assessing the welfare of hens.

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1.2. Thermal environment and heat stress in layer poultry farming

Heat stress is associated with physiological changes that compromise the productivity and animal welfare of laying hens. According to Rostagno *et al.,* (2020), adverse thermal environments affect metabolism and energy balance, highlighting the importance of proper environmental management in the laying hen production systems.

Recent studies have shown that heat stress significantly alters the physiological responses of hens to adverse environmental conditions. Kim *et al.,* (2024) reported that hens subjected to high levels of temperature and humidity for 28 days exhibited reduced feed intake and egg production, as well as changes in blood parameters, such as increased serum potassium and decreased sodium, indicating metabolic imbalances associated with environmental heat. In addition, higher temperatures increased the rectal temperature and water intake of the hens, suggesting physiological efforts to dissipate body heat. These results demonstrate that intense heat conditions impact both the physiology and productive performance of hens, highlighting the importance of thermal management in maintaining hens' homeostasis and productive efficiency (Kim *et al.,* 2024).

1.3. Ventilation systems and environmental control in poultry houses

Ventilation is a key factor in the distribution of temperature, humidity, and air speed in layer houses, and directly influences the homogeneity of the internal microclimate. Negative pressure ventilation systems, which are widely used in dark-house-type sheds, can present limitations in terms of environmental uniformity depending on their design and management. In contrast, the incorporation of positive pressure, either alone or in combination, promotes vertical air circulation and reduces thermal gradients (Hu *et al.,* 2024).

Computational fluid dynamics (CFD) modeling studies have shown that different ventilation configurations significantly alter airflow patterns and thermal stability in layer hen houses. In particular, ventilation systems with heat recovery have been evaluated as an alternative to improve the thermal stability of the indoor environment during winter while maintaining air renewal and environmental control (Song *et al.,* 2025).

1.4. Relationship between thermal comfort, behavior, and productive performance

Thermal comfort plays a central role in the interaction between environmental conditions, behavioral expression, and productive efficiency in laying hens. Beyond physiological responses, hens exposed to suboptimal temperatures exhibit behavioral adjustments, such as reduced feed intake, increased water consumption, and altered resting patterns, which directly affect energy allocation and production outcomes.

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Thermal comfort can directly influence the productive efficiency of poultry, as appropriate environmental conditions are associated with reduced energy expenditure for thermoregulation and favor the allocation of metabolizable energy towards growth and production (Baêta and Souza, 2010b). Recent reviews indicate that deviations from the thermoneutral zone compromise productive performance by inducing compensatory physiological and metabolic responses, thereby reinforcing the importance of environmental control in poultry facilities (Oliveira Junior *et al.,* 2021; Calderaro *et al.,* 2023).

2. METHODOLOGY

The research was conducted in the poultry sector of the Faculty of Agricultural and Veterinary Sciences at UNESP, Jaboticabal Campus, utilizing barns with positive and negative pressure ventilation systems, under average temperatures ranging from 21.6 to 34.4 °C and 22 to 25.3°C, respectively. In Group 1, the positive pressure system used a conventional barn with thermal control provided by fans, featuring a concrete floor, wire mesh, and no curtains. The barn measured 70 m in length and 4 m in width, oriented east-west with a fiber cement tile roof.

In Group 2, the negative pressure system, the shed had similar structural characteristics regarding floor and roof materials; however, it differed in width and environmental control configuration. The size was 70 m long, 8 m wide, and 2.20 m high. There were differences in ceiling height, use of polyethylene curtains, evaporative panels, and exhaust fans, characterizing the dark house system. In both groups, metal cages measuring 45 cm × 52 cm × 42 cm were used. The drinkers used were of the nipple type, with water available to the animals at all times. The metal feeders were placed in front of the cages, with one feeder for each experimental unit. The feed was replenished twice a day, in the morning and afternoon, and was provided *ad libitum*.

Twelve Bovans White laying hens, 50 weeks old, were used in each system. Two trained observers recorded laying hens' behavior using the scan sampling technique every 5 min over three consecutive days during the morning (9:00–9:30 a.m.) and afternoon (3:00–3:30 p.m.) periods. The frequency of each behavioral event was recorded according to a predefined ethogram. Although the number of laying hens per system was limited, repeated scan sampling increased the number of behavioral observations, improving descriptive robustness.

A predefined ethogram (Table 1), based on literature reviews such as Rudkin and Stewart (2003) along with preliminary observations, was used to categorize the behaviors. The behavioral categories presented in the ethogram below include both behaviors related to animal welfare (feather exploration, non-aggressive pecking, and idleness) and those associated with heat stress (open beak, open wings, and aggressiveness). This tool enabled the objective quantification of behaviors, ensuring the reliability and reproducibility of the data owing to the precise definition of behavioral categories and observer training.

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The experimental design was exploratory and descriptive, aiming to identify behavioral trends in hens subjected to different ventilation systems. The data were analyzed using descriptive statistics, with the calculation of the relative frequencies (%) of the observed behaviors, without the intention of performing statistical inferences.

Although the barns differed in width, cage dimensions, stocking density, management, and laying hens genetics were standardized. The study evaluated ventilation systems under real production conditions. Given its exploratory design, limited sample size, and short observation period, the results represent behavioral tendencies, and no inferential statistical analyses were performed.

Table 1. Ethogram adapted from Rudkin and Stewart (2003) for observing the behavior of laying hens housed in cages

EVENTS	DESCRIPTION
Eating	The hen ingests feed from the feeder.
Drinking	The hen drinks water from a drinker.
Feather exploration	The hen uses its beak to explore the plumage, both for maintenance and for investigation.
Non-aggressive pecking	The hen lightly pecks other laying hens, usually in the lower ventral region of the neck, back, base and tip of the tail, or abdomen.
Aggressive pecking	The hen forcefully pecks another laying hen, causing an aggressive or defensive reaction, directed at the upper region of the head and comb, or the lower dorsal region of the neck.
Stereotypy	Repetitive and apparently functionless acts, such as continuous pecking at the cage or troughs, scratching on top of other laying hens, among others.
Open beak	The act of remaining with the beak open and panting, promoting heat loss through respiration.
Open wings	The act of remaining with the wings semi-open, in order to increase body surface area for heat exchange by convection.
Idle	The hen shows no movement or apparently does not fit into any of the previous behaviors.

Source: Adapted from Rudkin and Stewart (2003).

3. RESULTS AND DISCUSSION

Table 2 shows the average temperatures inside the sheds during the three days of evaluation:

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Table 2. Average temperatures inside the sheds

	Group 1 - PPB		Group 2 - NPB	
	Morning	Afternoon	Morning	Afternoon
Day 1	19.2°C	31.8°C	21.5 °C	24.3 °C
Day 2	23.5°C	35.2°C	22.1°C	25.3°C
Day 3	22.1°C	36.1 °C	22.2°C	26.5°C
Overall average	21.6°C	34.4°C	22.0°C	25.3°C

PPB = Positive Pressure Barn; NPB = Negative Pressure Barn.
Source: Prepared by the authors.

Temperature variations were observed throughout the day in the shed of group 1, as well as between the two sheds. The productive performance of the laying hens can be severely affected when exposed to environmental temperatures above the ideal range (Nawab et al., 2018). According to the same author, egg production may decrease by up to 28.8% under these conditions, with consequences for laying frequency and egg quality, as evidenced by a reduction in shell thickness, decreased weight, and increased porosity.

The research did not evaluate egg production, but it is important to highlight that, since average temperatures were well above the ideal range (18°C to 28°C), and according to the Bovans White breed manual, laying performance is impaired when temperatures exceed 28°C. Behaviors associated with heat stress observed in the PPB are described in the literature as potentially related to reduced productive performance. Oliveira et al. (2014) and Silva et al. (2012) found in their studies that egg production at lower temperatures, 20°C and 26°C, was higher than at 32°C, revealing one of the consequences that inadequate temperatures can cause.

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Table 3. Behavioral events of laying hens in different ventilation systems

	Group 1-PPB: (Morning)	Group 1- PPB: (Afternoon)	Group 2- NPB: (Morning)	Group 2-NPB: (Afternoon)
Eating	55%	32%	57%	54%
Drinking	7%	20%	5%	3%
Feather exploration	14%	9%	9%	7%
Non-aggressive pecking	2%	0	0	2%
Lying down	12%	10%	12%	20%
Idleness	21%	15%	17%	12%
Stereotypy	1%	0	0	0
Open beak	0	12%	0	2%
Open wings	0	2%	0	0

PPB = Positive Pressure Barn; NPB = Negative Pressure Barn.
Source: Prepared by the authors.

In table 3, in the first column (group 1), predominantly "eating" behavior was observed. This result is consistent with that of Cordeiro *et al.,* (2011), who observed in their study that the hens spend most of their time eating or near the feeders. The main justification for the behavior is the spatial limitation imposed by the cages and the need for maintenance (Ferreira *et al.,* 2022).

The most frequent feeding behavior in both systems is related to the limited space in the cages (Argenta *et al.,* 2009). In addition, the hens exhibited exploratory and social interaction behaviors, such as feather exploration and pecking.

It is worth noting that, although present, behaviors associated with comfort, such as lying down, were not predominant in the hens behavioral repertoire during the evaluation of this period. Behaviors such as stereotypies and beak opening were observed less frequently, suggesting that the hens did not exhibit evident signs of discomfort during the morning observation period.

In the second column, feeding, as expected, also stood out as the most frequent activity. In all the results, it is observed that the variable “eating” had higher values; the main justification for this behavioral pattern is the spatial limitation imposed by the cages (Teixeira, 2017).

However, the analysis also revealed other relevant behaviors. It was observed that the hens exhibited stereotypy, feather exploration, and social interaction (non-aggressive pecking), indicating an environment that provided both appropriate and inappropriate stimuli for the hens welfare.

In group 1, during the afternoon period, the variations were greater compared to the morning. The data show that the activity "eating" continued to have the highest frequency, but with

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a significant reduction when compared to the previous period, corroborating Santana et al. (2018), who found that the decrease in feed intake was proportional to the increase in temperature.

The "drinking" behavior was observed with high frequency, which occurs at elevated temperatures. According to Guimarães *et al.,* (2014), when hens are exposed to high temperatures, their bodies have difficulty eliminating excess heat. Consequently, the hens' internal temperature rises, leading to a reduction in feed intake and an increase in water consumption.

This change in feeding behavior may compromise nutrient availability for egg production, since a large part of the ingested energy is directed towards thermoregulation mechanisms, to the detriment of egg production. Behavioral responses like these are described in the literature as potentially associated with a reduction in the quantity and quality of eggs (Nawab *et al.,* 2018).

The presence of social and exploratory behaviors, such as "feather exploring" and "non-aggressive pecking," indicated that the hens sought social interaction and environmental stimulation. However, during the afternoon period, the average temperature reached 34.4°C, with a peak of 36.1°C recorded, well above the recommended maximum of 28°C (Tinôco, 2001). During this period, the occurrence of stereotypical behaviors, such as "stereotypy" and "open beak," was higher.

In contrast, the shed of group 2, which on the same day and during the same period showed a maximum recorded temperature of 26.5°C, did not present behavioral signs of heat stress. It is suggested that the elevated temperatures may have contributed to thermal discomfort in hens from group 1, indicating the need for a more suitable temperature for the species' animal welfare. This is because this factor is considered the climatic element with the greatest influence on the physical environment of the animals (Amaral *et al.,* 2016) and may be one of the main factors responsible for the manifestation of behaviors considered inappropriate in hens, such as "aggressive pecking", "stereotypy", "open wings" and "open beak."

As in the previous results, in the fourth column (afternoon), group 2 also showed feeding as the predominant behavior. Value corresponds to the group with better temperature control. According to Oliveira (2014), hens under heat stress may decrease feed intake and increase water consumption. In this study, an increase in water consumption was observed in hens under heat stress, demonstrating a greater need for water at higher temperatures. This occurs because one of the functions of water in the organism is to help regulate body temperature (Leeson and Summers, 2001).

There was a difference in the value of animals lying down compared to group 1, and an absence of hens with open wings, indicating greater thermal comfort. In hotter climates, hens may open their wings to increase heat exchange with the environment (Baêta; Souza, 1998a).

However, the variable "feather exploration," which according to Barbosa Filho *et al.,* (2007) and Santos *et al.,* (2010) is also an indicator of comfort and natural activity, was observed less

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frequently compared to previous periods. The three-day evaluation period follows minimum standards commonly adopted in behavioral observational studies; however, longer-term assessments could provide additional insights into the consistency of the observed patterns.

Although physiological indicators (e.g., corticosterone levels and respiratory rate), productive performance parameters, and thermal comfort indices such as enthalpy or temperature–humidity index (THI) were not evaluated in the present study, their inclusion in future research could provide a more comprehensive understanding of the effects of different ventilation systems on hen welfare and productivity.

4. CONCLUSIONS

Negative pressure ventilation, combined with evaporative pads and exhaust fans, was associated with thermal conditions closer to the comfort range and with a lower frequency of stress-related behaviors under the evaluated conditions. This system showed a greater occurrence of behaviors commonly linked to comfort, such as lying down, without reducing feeding activity, whereas the positive pressure system was associated with more frequent heat-related responses, including open beak and increased water consumption. Within the exploratory scope of this study, the results suggest that the NPB system may represent a promising alternative for improving environmental conditions in laying hen production, highlighting the relevance of ventilation management for poultry welfare.

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REVISTA CIENTÍFICA - RECIMA21 ISSN 2675-6218

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