



BRUNA PONTARA VILAS BOAS RIBEIRO

**THERMAL ENVIRONMENT AND PHYSIOLOGICAL AND
PRODUCTIVE PARAMETERS IN LAYING HENS:
ASSESSMENT AND MODELING**

**LAVRAS - MG
2020**

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Tese apresentada à Universidade Federal de Lavras, como parte das exigências do Programa de Pós-Graduação em Engenharia Agrícola, área de concentração em Construções, ambiência e tratamento de resíduos, para a obtenção do título de Doutor.

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LAVRAS - MG

2020

**Ficha catalográfica elaborada pelo Sistema de Geração de Ficha Catalográfica da Biblioteca Universitária da UFLA,
com dados informados pelo(a) próprio(a) autor(a).**

Ribeiro, Bruna Pontara Vilas Boas.

Thermal environment and physiological and productive parameters
in laying hens: assessment and modeling / Bruna Pontara Vilas Boas
Ribeiro. – 2020.

118 p. : il.

Orientador: Tadayuki Yanagi Junior.

Coorientadores: Alessandro Torres Campos; Daniela Duarte de
Oliveira; Renato Ribeiro de Lima; Márcio Gilberto Zangeronimo.
Tese (Doutorado) - Universidade Federal de Lavras, 2020.
Bibliografia.

1. Avicultura de postura. 2. Ambiência Animal. 3. Inteligência
Artificial. I. Yanagi Junior, Tadayuki. II. Campos, Alessandro Torres.
III. Oliveira, Daniela Duarte de. IV. Lima, Renato Ribeiro de. V.
Zangeronimo, Márcio Gilberto. VI. Título.

BRUNA PONTARA VILAS BOAS RIBEIRO

**AMBIENTE TÉRMICO E PARÂMETROS FISIOLÓGICOS E PRODUTIVOS DE
POEDEIRAS: AVALIAÇÃO E MODELAGEM**

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**LAVRAS – MG
2020**

“Dedico esta tese aos meus pais, Carla Pontara e Afrânio Ribeiro, pelo imenso amor, paciência e pelo grande apoio dedicado em todos os momentos de dificuldade, sendo pilares que sustentam esse sonho. À minha querida irmã, Ana Clara Pontara, que me ensina a cada dia o que é ser feliz e que Deus está sempre presente entre nós. Ao meu estimado Uellington, pelo amor, carinho e força”.

DEDICO

AGRADECIMENTOS

A Deus, o precursor de todas as minhas idealizações, de toda a minha alegria, de um mundo em que, sem ELE, nada sou.

À Universidade Federal de Lavras, Departamento de Engenharia Agrícola (DEG), seu corpo docente, direção e administração, pela oportunidade de aprendizado a mim concedida.

À Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES), pelo apoio financeiro.

Ao meu orientador professor Dr. Tadayuki Yanagi Junior, e aos coorientadores professores Dr. Alessandro Torres Campos, Dra. Daniela Duarte Oliveira, Dr. Renato Ribeiro de Lima e Dr. Márcio Gilberto Zangeronimo pelo suporte, correções e incentivos constantes.

À banca avaliadora: professor Dr. Tadayuki Yanagi Junior, professora Dra. Fátima de Jesus Folgôa Baptista, professor Dr. Alessandro Torres Campos, professor Dr. Yamid Fabián Hernández Julio, professor Dr. Iran José Oliveira da Silva, professora Dra. Giselle Borges Moura e professor Dr. Leonardo Schiassi.

Agradeço em especial ao professor Dr. Antônio Gilberto Bertechini, pelo apoio com as dietas experimentais confeccionadas em sua granja. Ao grupo Aviário Santo Antônio (ASA), em especial ao Sr. Benedito Lemos de Oliveira e à Dra. Daniela Duarte de Oliveira, pelo apoio e suporte concedidos para a condução deste experimento.

Aos meus tesouros: Afrânio e Carla, por sempre me apoiarem em todos os meus projetos de vida, por me darem amor e carinho, e pela paciência sempre depositada, mesmo que em momentos difíceis. À minha irmã, Ana Clara Pontara, pessoa maravilhosa em minha vida, singular, meu amor maior.

Aos meus maravilhosos e queridos avós, Antônia Moreira e Carlos Pontara, pelo amor e apoio sempre presentes e de um valor enorme em minha vida. A minha tia, Grazielle Moreira, que, com sua docilidade, encanta a todos. E a minha madrinha, Roberta Moreira, que, mesmo à distância, proporciona-me todo o amor e me impulsiona a viver minhas conquistas intensamente. Ao meu querido Uellington Corrêa, pelo amor, companheirismo, gentileza e, principalmente, por fazer parte desta caminhada.

Agradeço também as colegas de departamento: Luana, Maria Alice e Bruna Amaral pelo apoio e amizade! Assim como, as grandes amigas conquistadas nestes cinco anos de UFLA: Tatyany, Marina, Evelyn e amigos dos grupos de estudo NESUI e NECTA.

A todos que estão envolvidos e que direta ou indiretamente contribuem para dias melhores, para uma meta a ser cumprida com êxito!

“As nuvens mudam sempre de posição, mas são sempre nuvens no céu. Assim devemos ser todo dia, mutantes, porém leais com o que pensamos e sonhamos; lembre-se, tudo se desmancha no ar, menos os pensamentos”. (Paulo Belecki)

RESUMO GERAL

Objetivou-se, com o presente estudo, avaliar e modelar matematicamente o efeito do ambiente térmico sobre as respostas fisiológicas de galinhas poedeiras. O estudo foi conduzido em quatro túneis de vento climatizados, onde noventa galinhas poedeiras em fase de pico produção, com idade de 28 semanas, foram alojadas individualmente em gaiola e submetidas a diferentes desafios térmicos. As aves foram submetidas à combinação fatorial de cinco temperaturas de bulbo seco do ar (t_{bs} : 20, 24, 28, 32 e 36 °C), dois níveis de umidade relativa do ar (UR: 40 e 60%) e três velocidades do ar (V_{ar} : 0,2, 0,7 e 1,4 m s⁻¹), totalizando 30 desafios térmicos. As respostas fisiológicas temperatura superficial ($t_{superficial}$), temperatura cloacal ($t_{cloacal}$) e frequência respiratória (FR) foram mensuradas em intervalos de 10 min durante cada teste experimental (desafio térmico), com duração mínima de três horas e máxima de seis horas. Modelos empíricos foram ajustados e modelos baseados em inteligência artificial (Rede neural artificial - RNA e sistemas *fuzzy*) foram desenvolvidos para predição das respostas fisiológicas de galinhas poedeiras em função das variáveis que caracterizam os diferentes desafios térmicos. Ademais, faixas de conforto térmico foram estabelecidas para as aves em função destas variáveis. Por meio da análise de agrupamentos, modelos empíricos e gráficos com faixas de conforto térmico para galinhas poedeiras *Hy Line*, pode-se inferir que, em um ambiente experimental controlado, as faixas de conforto encontradas, estão próximos aos especificados pela literatura. O desenvolvimento de modelos embasados em inteligência artificial, *fuzzy* e RNA, principalmente em função das variáveis climáticas, t_{bs} , UR, V_{ar} , obtiveram ajustes adequados para predição das variáveis fisiológicas $t_{cloacal}$, $t_{superficial}$ e FR.

Palavras-chave: Avicultura de postura. Ambiência Animal. Inteligência Artificial. Modelos Empíricos. Fisiologia de aves de postura. Faixas de conforto térmico.

GENERAL ABSTRACT

This study aimed to evaluate and model mathematically the effect of the thermal environment on the physiological responses of laying hens. The study was conducted in four air-conditioned wind tunnels, where ninety laying hens in peak production phase, aged 28 weeks, were housed individually in a cage and subjected to different thermal challenges. The birds were subjected to the factorial combination of five dry air bulb temperatures (t_{bs} : 20, 24, 28, 32 and 36°C), two levels of relative humidity (RH: 40 and 60%) and three air velocities (V : 0.2, 0.7 and 1.4 m s⁻¹), totaling 30 thermal challenges. The physiological responses surface temperature (t_{surface}), cloacal temperature (t_{cloacal}) and respiratory rate (RR) were measured at 10 min intervals during each experimental test (thermal challenge), with a minimum duration of three hours and a maximum duration of six hours. Empirical models were adjusted and models based on artificial intelligence (Artificial Neural Network - ANNs and *fuzzy* systems) were developed to predict the physiological responses of laying hens as a function of the variables that characterize the different thermal challenges. In addition, thermal comfort ranges have been established for birds as a result of these variables. Through the analysis of clusters, empirical models and graphics with thermal comfort bands for Hy Line laying hens, it can be inferred that, in a controlled experimental environment, the comfort bands found are close to those specified by the literature. The development of models based on artificial intelligence (*fuzzy* and ANNs) mainly due to the climatic variables, t_{bs} , RH, V , obtained adequate adjustments to predict the t_{cloacal} , t_{surface} and RR physiological variables.

Keywords: Laying poultry. Animal Welfare. Artificial intelligence. Empirical models. Poultry physiology. Thermal comfort thresholds.

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FIRST PART

1 INTRODUCTION

Laying hens are an important source of low-cost animal protein for many people around the world. However, poultry production requires more technological advances and studies to fill gaps in productivity. Currently, environmental issues, food biosafety and animal welfare are the three biggest challenges for poultry production. In this context, there is a gradual concern about the potential impacts of the thermal environment on the physiology, behavior and performance of laying hens due to temperature fluctuations inside poultry houses.

The effects of thermal stress on laying hens are associated with their susceptibility to heat stress and high metabolic heat production, in addition to their poor heat dissipation through convection and radiation due to feather density. Thermal stress is usually accompanied by reduced feed intake with a consequent reduction in productivity and egg production, production of small eggs, soft-shelled eggs, and the occurrence of cloacal prolapse.

Exposure of birds to high temperatures leads to heat stress and triggers physiological and regulatory mechanisms in an attempt to maintain homeostasis. Therefore, characterizing the thermal environment inside and outside poultry houses is crucial to define strategies to mitigate the effects of climate on animal production.

Environmental conditions outside livestock buildings and its internal microclimate have direct and indirect effects on all stages of animal production and may result in reduced thermal comfort, welfare, and productivity, with consequent socio-economic losses.

Technological advances in animal environment have allowed mitigating unwanted low-production scenarios. Moreover, the search for ethical and high-quality products by society and the international market has contributed to progress in animal production. Artificial intelligence methodologies such as fuzzy logic and artificial neural networks (ANNs) can generate results that positively impact the productivity and systematization of information valuable to decision-making.

Therefore, the hypothesis drawn for this work is that quantify thermal comfort thresholds and develop artificial intelligence based-model can increase the precise control over thermal environment conditions for laying hens. The aim of the thesis was to evaluate

and model mathematically the effect of the thermal environment on the physiological responses of laying hens that was used to establish thermoneutral zones.

2 REVIEW OF THE LITERATURE

The poultry industry provides significant supply of animal protein and has great potential for egg production, therefore requires technological advances and studies in order to fill gaps surrounding higher productivity in the poultry chain. In this context, the thermal environment control is essential to obtain adequate performance indexes.

The present theoretical framework addresses the control of the thermal environment inside commercial aviaries, and its interference in the physiology and performance of animals, making it essential for the definition of the production system, type and structure of the installation and management techniques to be used performed to minimize the negative effects of thermal stress on birds and the maintenance of their homeostasis. It also addresses the use of computational modeling techniques, such as intelligent expert systems, which are capable of performing tasks or solving problems from a knowledge base. It is an alternative that aims to quantify the interaction of indirect measures, such as air temperature, humidity relative ventilation, among other environmental variables, and thus establish more objective criteria in the decisions of producers.

2.1 Homeothermy and thermal environment in laying hens

The concept of thermal comfort is based on thermal environmental components and analysis of environmental conditions as a function of the thermoneutral zone of the species and its physiological characteristics, which regulate internal body temperature in animals (BRIDI, 2010).

In laying hens, the ideal ambient temperature ranges from 21 to 28°C (CASTILHO et al., 2015), although some authors report a thermoneutral zone ranging from 20 to 24°C for hens at laying period (CHEPETE; XIN, 2000; YANAGI et al., 2011). According to Donald (1998), well-feathered birds under environments with a temperature of 26.7°C and relative humidity of 60% are near the upper limit of their comfort zone, i.e., they are not heat-stressed. However, environments with similar temperatures but with relative humidity above 80% become uncomfortable for laying hens, impairing their performance (JÁCOME et al., 2007).

Birds maintain approximately constant body temperature (41.1°C) under thermal comfort conditions; they are sensitive to thermal variations and may have breeding problems under conditions of environment fluctuations (ALBINO et al., 2014). In birds, the body temperature remains approximately constant when air temperature rises gradually up to 33°C

(CAMERINI et al., 2016). The amount of thermal energy stored per unit of body mass depends on the bird's body temperature and can be increased or decreased by thermogenesis and thermolysis processes (CASTILHO et al., 2015).

2.2 Thermal environment: air temperature, relative humidity, and wind speed

Air temperature has a direct effect on livestock since short-term fluctuations in air temperature promote changes in the behavior and physiology of animals. However, the air temperature itself is not sufficient to predict the thermal sensation of an animal over time. Therefore, it must be associated with relative humidity because different values of temperature and relative humidity produce contrasting thermal sensations, resulting from or not in thermal stress (BARACHO et al., 2013).

Temperature and relative humidity are the two environmental variables of most significant influence on thermal comfort in animals. At high temperatures, the primary mechanism of heat dissipation in birds is evaporation, which is highly dependent on relative humidity (BAËTA; SOUZA, 2010). Overall, birds can alter blood flow between core and skin and change the evaporation rate from the respiratory tract to control heat losses (CASTILHO et al., 2015; ABREU et al., 2007).

Ventilation inside poultry houses helps maintain air quality with the least possible exchanges, allowing the removal of gases from bird excreta while aiding heat dissipation. Recommended air renewal levels vary with the age of animals.

Tinôco (2001) stated that birds are continually exchanging heat with the thermal environment, and this exchange is efficient when the air temperature is within limits. These limits are dependent on the thermal sensation, which is encompassed by temperature, humidity and wind speed inside the poultry building. According to Miragliotta et al. (2006), the thermal sensation is closely related to the airflow across the surface of the birds' body, facilitating heat dissipation to the environment.

2.3 Thermal Comfort Index

Thermal comfort indices are used as a reference for evaluating the thermal environment in livestock and consider the following environmental parameters: air temperature, relative humidity, velocity, and radiation. Each parameter has a particular weight value based on its relative importance to the animal.

2.3.1 Temperature and humidity index (THI)

The temperature and humidity index (THI) was developed by Thom (1958) and associates dry-bulb and wet-bulb temperatures. It was initially used for cattle by official United States climatology agencies, but nowadays, it has been widely used for several species.

According to Jácome (2009) and Takahashi et al. (2009), THI values below 70 are considered comfortable for domestic animals, whereas values above 78 are considered stressful. Vitorasso and Pereira (2009) stated that although THI does not consider the effects of radiation, it can be used to predict thermal comfort under conditions where black globe temperature is not available.

Climatological information commonly available in meteorological stations and databases obtained from satellite images provide the basis for bioclimatic mapping (YANAGI JUNIOR, 2006). With this information, it is possible to calculate the THI, which encompasses information on temperature and relative humidity for the characterization of certain bioclimatic zones.

Each mesoregion has a different bioclimate, with distinct THI values inside and outside the poultry building. According to the findings of Barbosa Filho (2004) for laying hens, a THI from 71 to 75 is considered normal, 75 to 84 is danger status, and a THI from 84 to 87 is an emergency.

The temperature and humidity index (THI) is calculated based on Equation 1 (THOM, 1958):

$$\text{THI} = t_{\text{db}} + 0.36 \cdot t_{\text{dp}} + 41.5 \quad (1)$$

where:

t_{db} : air dry-bulb temperature (°C)

t_{dp} : dew-point air temperature (°C)

2.3.2 Black globe-humidity index (BGHI)

Buffington et al. (1981) proposed the use of black globe-humidity index (BGHI) as a single parameter to evaluate the effects of dry-bulb temperature, air humidity, radiation level,

and air movement. It is considered the most appropriate thermal index to evaluate thermal comfort in conditions where animals are exposed to solar radiation.

According to the literature for broiler chickens, environments with temperatures ranging from 16 to 20°C have a BGHI from 59 to 67 (cold environment), while environments with temperatures around 26°C have a BGHI varying from 69 to 77 (comfortable environment). Environments with temperatures ranging from 32 to 36°C have a BGHI fluctuating from 78 to 88 (warm environment, thermal stress) (MEDEIROS et al., 2005). Other authors reported that the lower and upper limits of thermal comfort for commercial broilers based on BGHI, with t_{db} ranging from 15°C to 26°C (BAÊTA; SOUZA, 2010) are between 69.1 and 77.5, respectively (OLIVEIRA NETO et al., 2000).

The BGHI is calculated by equation 2 (BUFFINGTON et al., 1981):

$$BGHI = t_{bg} + 0.36 \cdot t_{dp} + 41.5 \quad (2)$$

where:

t_{bg} : black-globe temperature (°C)

t_{dp} : dew-point air temperature (°C)

2.3.3 Enthalpy (H)

Enthalpy is defined as the amount of energy of moist air per unit mass of dry air ($\text{kJ kg}_{\text{dry air}}^{-1}$) and correlates temperature and humidity in the environment, indicating the amount of energy contained in a water vapor mixture. According to Vieira et al. (2010), enthalpy measures the total amount of energy in the air, including not only the energy of the closed system but also the energy exchanged with its surroundings.

In laying poultry, enthalpy is a thermodynamic property that helps to determine the thermoneutral zone, and thus the thermal comfort ranges for laying hens (BARBOSA FILHO et al., 2007). Energy expenditure is considerably higher under conditions of high temperatures; thus, energy is dissipated as heat, negatively influencing egg production and quality.

Several comfort ranges based on H is found in the literature for laying hens: 58 and 68.8 $\text{kJ kg}_{\text{dry air}}^{-1}$ (VIEIRA et al., 2010), 36 to 66 $\text{kJ kg}_{\text{dry air}}^{-1}$ (ALVES, 2006; NAZARENO et al., 2015). VIEIRA et al. (2010) developed tables of comfort ranges based on enthalpy for laying hens, with values ranging from 50 to 68.80 $\text{kJ kg}_{\text{dry air}}^{-1}$.

Enthalpy is calculated by equation 3 (ALBRIGHT, 1990):

$$H = 1.006 \cdot t_{db} + W \cdot (2501 + 1.805 \cdot t_{db}) \quad (3)$$

where:

t_{db} : air dry-bulb temperature (°C)

W: humidity ratio ($\text{kg}_{\text{water vapor}} \text{kg}_{\text{dry air}}^{-1}$)

$$W = \frac{(0.622 \cdot e_a)}{(P_{\text{atm}} - e_a)} \quad (4)$$

where:

e_a : water vapor partial pressure (kPa)

P_{atm} : atmospheric pressure (kPa)

2.3.4 Radiant heat load (RHL)

Another indicator of thermal environmental conditions is the radiant heat load (RHL). It is used to express the total radiation received by the black globe from all surroundings under steady conditions (ESMAY, 1982).

The farmer should pay close attention to the RHL incident on the roof and also the RHL inside poultry buildings; thus, roofs with high emissivity should be used to reflect the radiation received from the atmosphere. Concomitantly, the roof material should have high thermal inertia, absorbing the external heat, and delaying the heat transfer to the interior of the building (JACOME et al., 2017).

Therefore, roof materials can reduce heat amplitude inside poultry buildings. For example, roofing underlayment improves the thermal comfort of birds by reducing thermal transmission and increasing the thermal inertia, resulting in an environment with lower RHL (ABREU et al., 2012).

Tinôco (2001) highlights ventilation, misting, and sprinkling systems as strategies to minimize the effects of RHL so that indoor temperatures remain within thermoneutral ranges for birds. Those systems can keep RHL relatively homogeneous compared with outdoor conditions, especially on warmer days.

The RHL can be calculated by equation 5 (ESMAY, 1969).

$$\text{RHL} = \sigma \cdot (\text{MRT})^4 \quad (5)$$

where:

σ : Stefan-Boltzmann constant ($5.67 \times 10^{-8} \text{ W m}^{-2} \text{ K}^{-4}$);

MRT: mean radiant temperature (K).

The mean radiant temperature (MRT) is the uniform temperature of a surrounding surface giving off blackbody radiation (in order to eliminate the reflection effect), in which an occupant would exchange the same amount of heat by radiation as in the actual nonuniform environment (BOND; KELLY, 1955). The MRT can be expressed by Equation 6:

$$\text{MRT} = 100 \cdot \sqrt[4]{2.51 \cdot \sqrt{V_{\text{air}}} \cdot (T_{\text{bg}} - T_{\text{db}}) + \left(\frac{T_{\text{bg}}}{100}\right)} \quad (6)$$

where:

MRT: mean radiant temperature (K);

V_{air} : wind speed (m s^{-1});

T_{bg} : black-globe temperature (K)

t_{db} : air dry-bulb temperature (K).

2.4 Physiology of heat stress

2.4.1 Thermal comfort zone

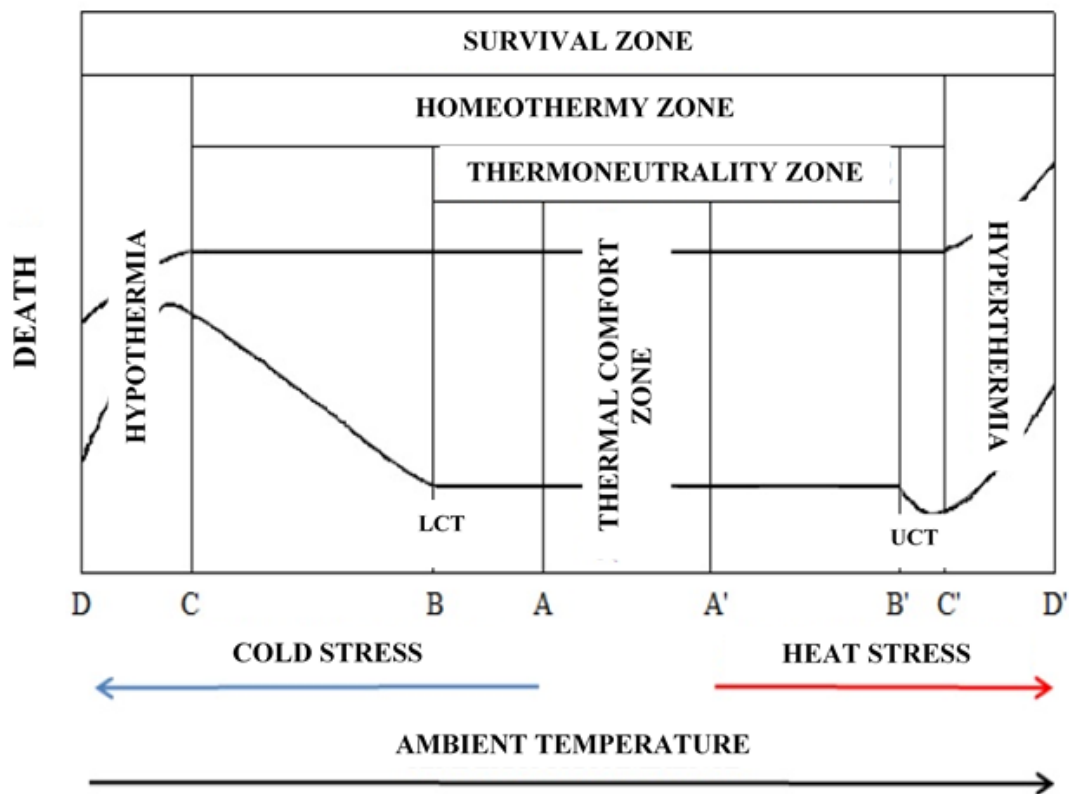
Animals breed in suitable environments can express their full genetic potential. Birds maintain approximately constant body temperature (homeothermy) with minimal metabolic rate and the lowest possible energy expenditure. This condition is defined as the thermal comfort zone (TCZ) or thermoneutrality zone. In the TCZ, the proportion of metabolizable energy for thermogenesis is reduced, while the net energy intended for body gain is increased.

Costa et al. (2012) stated that the proportion of metabolizable energy for thermogenesis in the thermal comfort zone is minimal while the net energy is maximum. The TCZ is dependent on several factors. Some of them are related to the animal, such as body weight, age, physiological status, group size, feeding level, genetics, while others are related to the environment, such as temperature, wind speed, and relative humidity. For a given environmental temperature range, birds maintain constant body temperature with minimal effort through thermoregulatory mechanisms.

As shown in Figure 1, the animal needs to produce body heat to maintain thermal equilibrium when the ambient temperature is below the comfort temperature. On the other hand, when the ambient temperature is above the thermal comfort zone, there is need to dissipate body heat to the environment. In both scenarios, the energy necessary for thermal maintenance (energy for maintenance) will be higher, reducing the amount of energy available for production (COSTA et al., 2012).

Below the lower critical temperature (LCT) identified by letter B in Figure 1, the body is unable to compensate for heat losses; above the upper critical temperature (UCT), identified by letter B' in Figure 1, the animal is unable to prevent the elevation of its internal temperature, resulting in hypothermia or hyperthermia, respectively (BRIDI, 2010).

Figure 1 - Schematic representation of effective ambient temperatures.



Source: Adapted from Costa et al. (2012).

2.4.2 Thermoregulatory mechanisms in laying hens

According to Ruzal et al. (2011), some physiological responses that explain the direct relationship between ambient temperature and surface temperature of birds may be related to the redistribution of blood flow, since heat exposure induces peripheral vasodilation.

Several physiological responses are triggered an attempt to increase heat dissipation by birds when ambient temperature increases (ALTAN et al., 2003), including respiratory rate, cloacal temperature, metabolic rate, reduced feed intake, and laying rate. Birds under thermal stress can increase their respiratory rate and thus increase body heat dissipation through the respiratory tract. Garcia et al. (2015) reported that the respiratory rate of laying hens ranges from 23 movements per minute (mov. min^{-1}) in a thermoneutral environment (20°C) to 273 (mov. min^{-1}) at high temperatures (35°C).

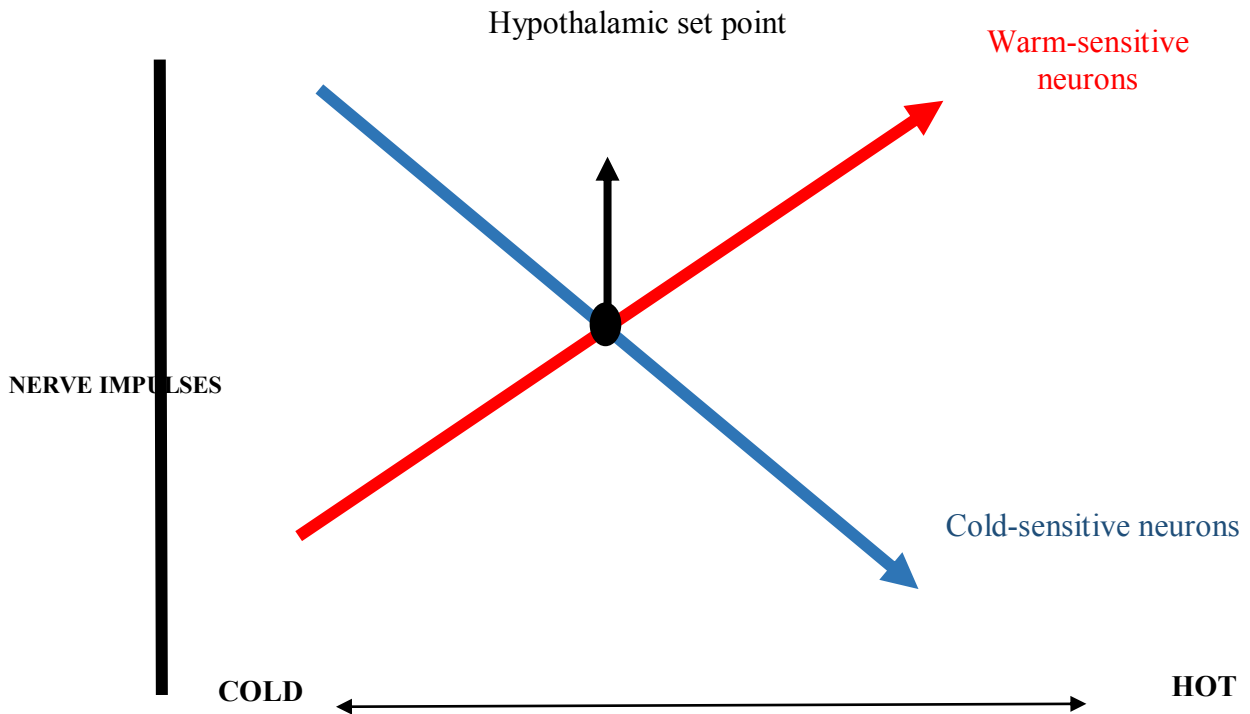
Increased cloacal temperature is a physiological response to conditions of high temperature and relative humidity, resulting in the storage of metabolic heat (SILVA et al., 2003). Previous research indicates that the increase of body temperature in birds is associated with the increase of ambient temperature; thus, infrared thermography can be used to evaluate the surface temperature and study thermal comfort in animals. For cloacal temperature, the thermal comfort may vary from 41 to 42°C (OLIVEIRA et al., 2006).

Besides reducing birds' performance, high ambient temperatures also induce hyperventilation. It causes excessive loss of carbon dioxide (CO_2), which is essential in the formation of calcium carbonate in the eggshell (JÁCOME et al., 2007). Hyperventilation leads to significant losses of CO_2 at the level of the pulmonary alveoli, increasing blood pH, and triggering respiratory alkalosis. Moreover, it reduces the concentration of diffusible calcium in the blood as well as calcium intake, which affects its deposition in the eggshell. There is also a reduction in the conversion of vitamin D3 to its metabolically active form, which is essential for the absorption and utilization of calcium by the body (PLAVNIK, 2003).

Abreu and Abreu (2012) reported that birds have low efficiency in high-temperature conditions since 80% of the ingested energy is used to maintain homeostasis, whereas only 20% is used for production.

The physiological temperature in homeothermic animals is controlled by the thermoregulatory center, which is located in the hypothalamus in the brain. According to Souza; Batista (2012), thermoreceptors distributed across the body surface of animals have thermoreceptive-specific cells that are activated by nerve impulses. This thermoregulatory network is integrated with hypothalamic neurons that respond to cold or heat. The synergy between warm-sensitive and cold-sensitive thermoreceptors constitutes the hypothalamic set point, which is responsible for the control of body temperature, working like a thermostat (FIGURE 2).

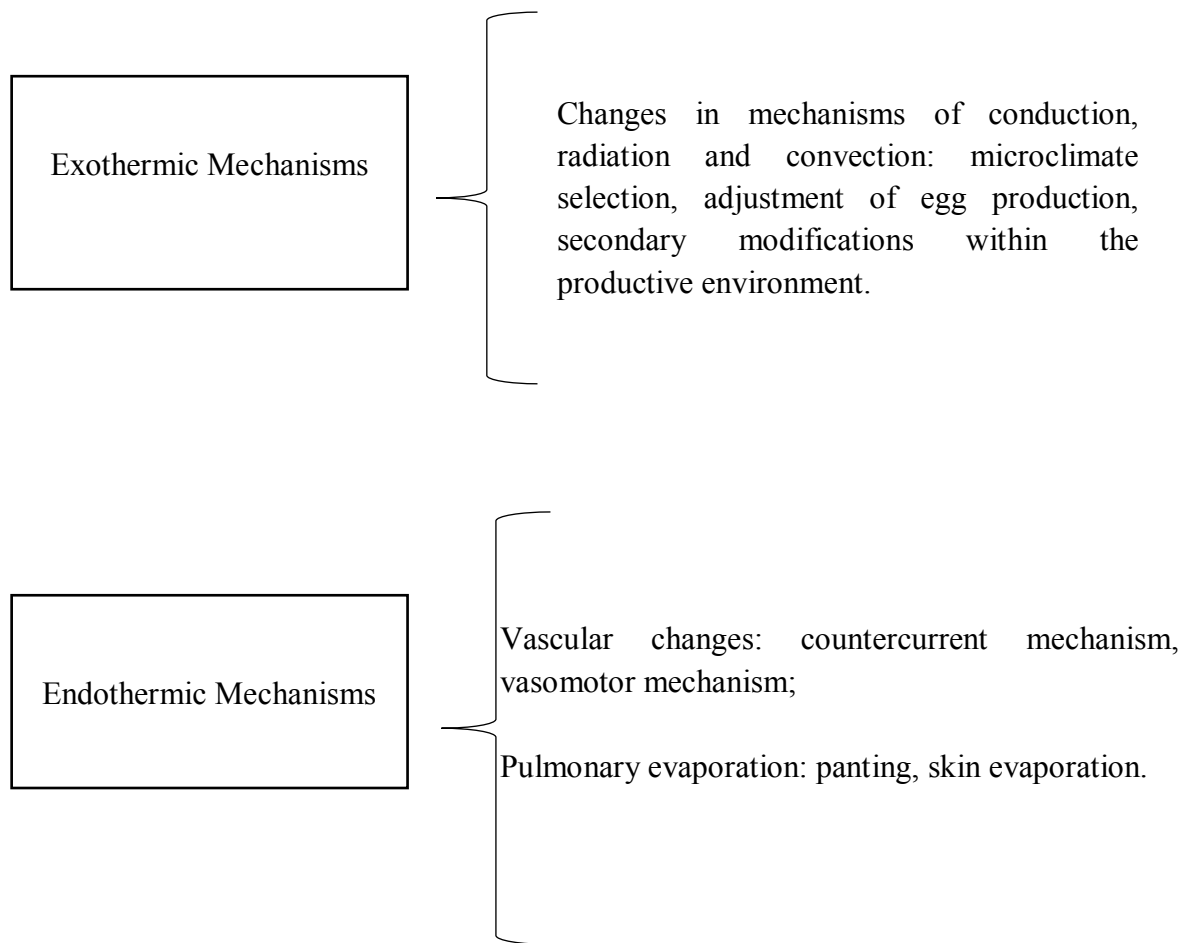
Figure 2 - Scheme of a hypothalamic set point in homeothermic animals.



Source: Adapted from Ferreira (2016).

The hypothalamus controls heat production and dissipation by several mechanisms. The skin blood flow, respiratory rate, and metabolic rate of animals are altered under conditions of heat stress (ALBINO et al., 2014). Behavioral mechanisms are the first to be activated by birds exposed to extreme temperatures. Birds touch the chest to the floor, spread their wings to increase the contact surface and ventilation across feathers and reduce the feed intake (low heat-increment diets may be used in sweltering conditions). Under extreme conditions, birds may remove their feathers to favor heat dissipation (Figure 3).

Figure 3 - Flowchart of physiological responses of birds under heat stress.



Source: Elaborated by the author (2019).

2.5 Physiology of heat stress applied to egg production

Birds maintain an approximately constant internal temperature ranging from 40°C to 43°C, making them homeothermic animals. However, the environmental condition must be controlled to avoid adverse effects on laying hen performance (MACARI; FURLAN, 2001).

High ambient temperatures can be a significant stressor to laying hens and are associated with significant economic losses in the poultry industry. Therefore, ambient temperature should be considered by farmers when structuring the design of poultry buildings, aiming at reducing the risk exposure of birds to temperature and humidity peaks (ALBINO et al., 2014).

The impacts of high temperatures inside poultry houses include reduced growth rate (BOTTJE; HARRISON, 1985), immunosuppression (YOUNG, 1990) and high mortality

(YAHAV et al., 1996), thus leading to reduced productivity. The microclimate conditions indirectly influence the internal quality of eggs. During heat stress, birds tend to reduce feed intake and, consequently, egg weight is negatively affected (FREITAS et al., 2017).

The maximum performance is achieved by animals kept in a thermoneutral environment, i.e., when the dietary energy content is not used to compensate for thermal fluctuations (CIGR, 2002).

The temperature inside poultry houses for adult birds ranges from 15°C to 28°C, with relative humidity between 40% and 80% (FERREIRA, 2016). The ideal ambient temperature for egg production would range from 21°C to 26°C. In the temperature range from 26°C to 29°C, a slight reduction in egg size may occur, whereas between 29°C and 32°C a significant loss in egg size is reported, negatively affecting production. Under temperatures between 35°C and 38°C, birds show clinical signs of prostration, and production may be severely affected (ALVES et al., 2007).

Under conditions of high ambient temperature, the most common heat dissipation mechanisms used by the bird include increased respiratory rate (hyperventilation), and peripheral vasodilation through non-evaporative losses (RODRIGUES, 2006; BROSSI et al., 2009).

Physiological processes are activated to maintain body homeothermy under heat stress conditions. Those mechanisms include peripheral vasodilation, which is necessarily accompanied by visceral vasoconstriction (to maintain blood volume). This mechanism is mediated by sympathetic vasoconstrictive nerves and can be triggered by the heating of blood vessels. These reactions are controlled by sympathetic nerves that originate from the medulla oblongata. Circulating catecholamines also increases peripheral vasoconstriction (BIAZZANOTTO et al., 2006).

Andrade et al. (2017) reported that the birds' body surface is covered by the feathers, which are extremely important in regulating the thermal balance when birds are exposed to cold environments. During periods of heat stress, the featherless areas, such as the comb, the dewlap, and the feet, are usually vasodilated.

2.6 Fuzzy logic for prediction of poultry environments

Technological advances in animal ambience have allowed mitigating unwanted low-production scenarios. Novel methodologies and technologies have been used to control the housing environment, which was generally based on empirical analyses of temperature and

relative humidity. According to Schiassi et al. (2015), the use of computational modeling techniques that are able to perform tasks or solve problems from a knowledge base, such as intelligent expert systems, is essential to quantify the interaction between indirect measures, including ambient temperature, relative humidity, ventilation, among other environmental variables, and thus establish more objective criteria in farmers' decisions.

The fuzzy set theory can be used to predict physiological responses and characterize environmental stressors, i.e., how ambient temperature interferes with body temperature and productivity (LACEY et al., 2000; AMENDOLA et al., 2004).

Fuzzy logic can also be incorporated into environmental control systems for activating fans, evaporative cooling systems, and warning signals. Therefore, it can avoid exposure to a stressful environment and also reduce economic losses (CASTRO et al., 2012).

Fuzzy systems have been used for prediction of comfort and welfare in confined animals (PANDORFI et al., 2007; FERREIRA et al., 2010; FERRAZ et al., 2017; ABREU et al., 2015; TAVARES et al., 2016), estrus in dairy cows (FERREIRA et al., 2007), physiological responses (FERREIRA et al., 2012; HERNÁNDEZ JULIO et al., 2015; ABREU et al., 2015) and productive responses of animals (PERISSINOTTO et al., 2009; SANTOS et al., 2009; SCHIASSI et al., 2008; TOLON et al., 2010). This methodology has been also used for controlling ventilation and evaporative cooling systems (PEREIRA et al., 2008) and analyzing production costs (NÄÄS et al., 2010).

The use of fuzzy systems in solving control problems started with the pioneering work of Mamdani (1973), inspired by Zadeh's work. Mamdani's fuzzy inference method combines the degrees of relevance for each of the input values by the minimum operator and adds the rules with the maximum operator.

The rules of this inference method are aggregated through the logical operator OR, modeled by the mathematical operator \vee and, for each rule, the logical operators AND and THEN are modeled by the minimum operator \wedge (PEDRYCZ; GOMIDE, 1998).

This method was based on only two generic rules, which have two inputs and one output (CREMASCO, 2008):

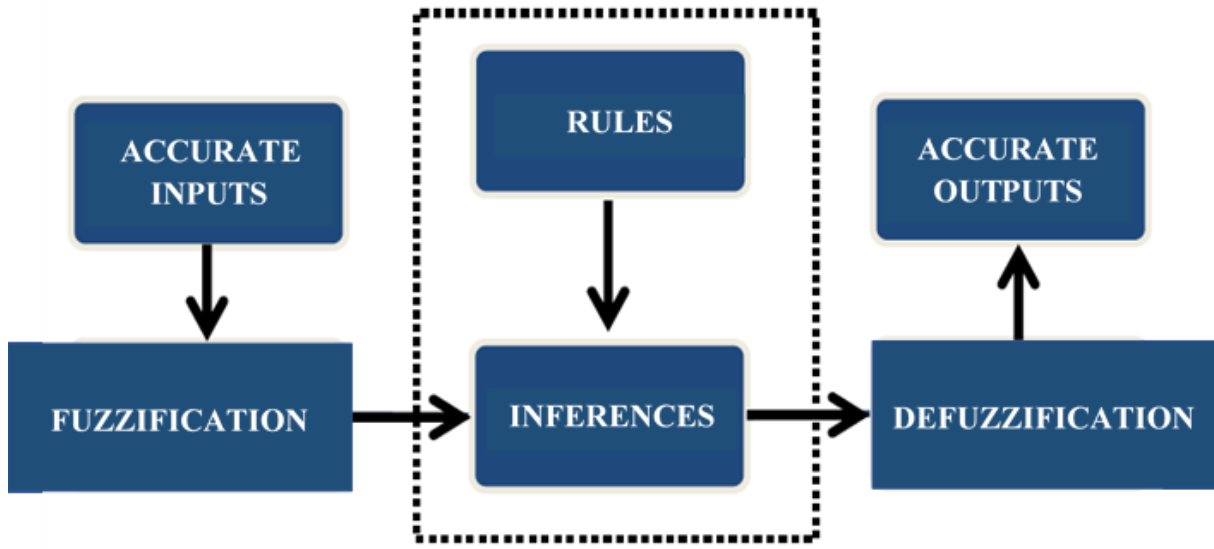
R1: IF x is A_1 AND y is B_1 THEN z is C_1 ;

R2: IF x is A_2 AND y is B_2 THEN z is C_2 ;

where A_i , B_i , C_i are fuzzy sets.

The process of building fuzzy model is presented in three steps: fuzzification (converts input values into fuzzy values), inference (determines output values based on predefined rule systems) and defuzzification (converts fuzzy values into numerical values) (BARROS; BASSANEZI, 2006; TAVARES; SCHIASSI, 2016) (FIGURE 4).

Figure 4 - Scheme of a fuzzy system.



Source: Abreu et al., 2015.

2.7 Artificial neural networks (ANN)

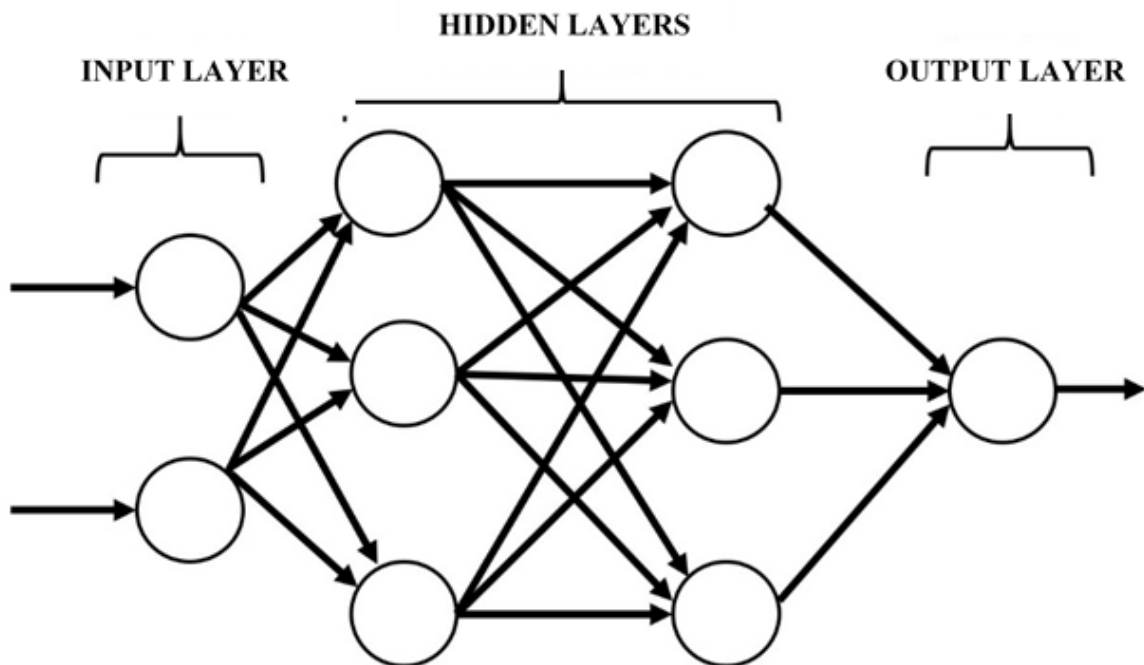
ANNs are nonlinear parametric models or parallel systems composed of artificial neurons, which are processing units interconnected via mathematical activation functions (BRAGA et al., 1998). These models are based on the functioning of the human brain and are therefore able to learn from experience (SILVA et al., 2010). A typical ANN architecture consists of at least three layers of nodes: an input layer, a hidden layer and an output layer (network's response). This form of the neural network is called a multilayer perceptron (MLP), in which the input and output layers are formed by the input-output dataset presented to the ANN (HAYKIN, 2001; BEALE et al., 2016).

According to Savegnago et al. (2011), the study of ANN considers nonlinear relations between input and output information. The advantages of ANNs include knowledge plasticity to changing inputs and outputs, fault tolerance, and interpolation capabilities (ZHANG et al., 2007).

Savegnago et al. (2011) further stated that ANNs can learn patterns of a dataset during the training process, thus providing consistent predictions or generalization capabilities over test sets. ANNs have a high computation rate provided by massive parallelism, so real-time processing of enormous data sets becomes feasible with the proper hardware (BISHOP, 1995).

ANNs have been used for prediction of body gain in animals (ROUSH et al., 2006; AHMADI et al., 2007; LOPES et al., 2017), environmental control of poultry houses (SILVA et al., 2013), prediction of physiological and productive responses (SCHIASSI, 2011; LOPES et al., 2014; ABREU et al., 2015), among other variables (FIGURE 5).

Figure 5 - Neural network with two hidden layers.



Source: Elaborated by the author (2019).

3 FINAL CONSIDERATIONS

The control of the thermal environment inside commercial poultry houses and its interference with the physiology and performance of animals becomes paramount in choosing the most suitable production system, building type and structure, and management techniques for minimizing the adverse effects of the thermal stress on laying hens and maintaining their homeostasis.

Computational methodologies based on artificial intelligence, such as fuzzy logic and artificial neural networks, are directly linked to the productivity and management in poultry and can minimize the effects of high air temperatures inside poultry houses.

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SECOND PART - ARTICLES

**ARTICLE 1 - THERMONEUTRAL ZONE FOR LAYING HENS BASED ON
ENVIRONMENTAL CONDITIONS, ENTHALPY, AND THERMAL COMFORT
INDEXES**

(DRAFT VERSION)

Article prepared for journal submission *Journal of Thermal Biology*.

Thermoneutral zone for laying hens based on environmental conditions, enthalpy and thermal comfort indexes

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Thermoneutral zone for laying hens

ABSTRACT: Controlling environmental conditions inside systems laying hens buildings and their effects on physiology and performance is essential in defining management strategies to alleviate the adverse effects of thermal stress in laying hens. Thus, we estimated thermoneutral zones for laying hens exposed to different heat-challenging conditions based on environmental conditions, enthalpy, and thermal comfort indexes. Clustering analysis, empirical models, and graphs were used to estimate thermoneutral zones for laying hens based on environmental conditions, enthalpy and thermal comfort indexes, and compare them with data available in the literature. The thermoneutral zones characterizing homeostasis for laying hens based on respiration rate (RR) are as follows: from 25.9 to 29.9°C for air dry-bulb temperature (t_{db}), from 67 to 75 for temperature-humidity index (THI), from 68 to 73 for black globe-humidity index (BGHI), from 45 to 56 kJ kg dry air⁻¹ for enthalpy (H) and 441.7 to 465.6 W for radiant heat load (RHL). Comfort limits for physiological responses cloacal temperature (t_{clo}), surface temperature (t_{sur}) and RR found in this study are 39.4 to 39.9 °C, 26.5 to 29.9 °C and 30 to 67 mov. min⁻¹, respectively. The number of repetitions and the use of mathematical modeling to be worked on, may directly impact the amplitude of each limit to be established for each variable of interest.

Keywords: thermal environment, egg-laying poultry, thermal comfort ranges, empirical models

HIGHLIGHTS

This study used physiological responses from laying hens to evaluate thermoneutral zones, depending on environmental conditions and thermal comfort indexes. Fluctuations in air temperature directly affect the physiology of laying hens. In high temperature environments, the increase in respiratory rate is the first thermoregulatory mechanism to maintain homeothermy. The establishment of new thermoneutrality limits in this study, allows a better management of the microclimate within the facilities, with emphasis on the system's productivity, in addition to updating the thermal comfort bands found in the literature.

1. INTRODUCTION

In laying hen production systems, farmers usually focus on animal productivity rather than welfare to infer thermal comfort status. Currently, environmental issues, food biosafety, and animal welfare are the three biggest challenges for poultry production. In this context, there is a gradual concern about the potential impacts of the environment on the behavior and performance of animals.

The synergism between thermal environment and heat production in laying hens can be explained through thermal comfort limits, which use physiological variables to characterize and evaluate poultry productivity. Several methodologies can be used for analyzing these thermal comfort ranges, including the thermoneutral zone (TNZ), which specifies the range of ambient temperatures (AT) where energy requirement is minimal and constant. Therefore, TNZ is an important parameter when adjusting the temperature inside poultry buildings. However, the TNZ of birds is associated with their body weight and age (Meltzer, 1987). Birds' ability to dissipate heat decreases as ambient temperature and relative humidity rise above the TNZ. As a result, the bird's body temperature rises, resulting in heat stress (Curto et al., 2007).

The maintenance of thermal balance inside commercial poultry buildings is directly related to the bird's productivity. In thermoneutral environments, in which temperature, relative humidity and air velocity are within the thermoneutral zone, a bird's productivity reaches its maximum. Under these conditions, the dietary energy is not diverted to compensate thermal deviations from the thermoneutral range (Ponciano et al., 2011).

Birds are homeothermic animals and produce heat to maintain relatively constant body temperature. Slight variations within their body temperature range are tolerated without significant disturbance (St-Pierre et al., 2003).

In laying hens, the ideal ambient temperature ranges between 21 and 28°C (Castilho et al., 2015). Ferreira (2016) reported that the productivity of laying hens increased under environments with relative humidity ranging from 40 to 70%.

Under conditions of high temperature, the normal respiratory rate of birds (23 mov. min⁻¹) may increase by up to tenfold in response to thermal discomfort, reaching up to 273 mov. min⁻¹ (Kassim and Sykes, 1982), indicating necessity the use of the evaporative mechanism of cooling (Santos et al., 2006). Under conditions of high air temperatures and high relative humidity, birds may develop respiratory alkalosis (Borges et al., 2003).

Due to the thick insulation provided by the feather coat on most of the body surface, sensible heat loss is more efficient in featherless areas, where blood flow increases when birds are exposed to heat stress. Laying hens do not have sweat glands and cannot perspire. Thus, birds lose excess heat mainly by evaporation (breathing) and through surfaces such as comb, wattle, shanks, and featherless areas below the wings.

Increased cloacal temperature is a physiological response to conditions of high air temperature and relative humidity, resulting from the storage of metabolic heat (Silva et al., 2003). For cloacal temperature, the thermal comfort may vary from 41 to 42°C (Oliveira et al., 2006). In this way, sensible exchanges between the animal and the environment are highly efficient; thus, the larger the difference between air and the bird's surface temperatures, the more efficient these exchanges will be (Nascimento and Silva, 2010).

Tinôco (2001) stated that birds are continually exchanging heat with the environment, and this exchange is efficient if the ambient temperature is within limits. These limits are dependent on the thermal sensation, which encompasses temperature, humidity and wind speed inside the building. The thermal sensation is closely related to the air flow across the surface of the birds' body, facilitating heat dissipation to the environment (Miragliotta et al., 2006). Therefore, these physiological mechanisms of body heat loss will be activated depending on the thermal environment surrounding the animal; thus, they are directly related to environmental variables (air temperature, relative humidity and velocity).

The approach of this work has a practical and direct effect on the analysis of yield losses due to thermal fluctuations in commercial laying hens, with updating of the thermal comfort limits for some variables that model the microclimate inside the facilities. Thus, this study used the physiological responses of laying hens to evaluate the thermoneutral zones, depending on environmental conditions, thermodynamic property and thermal comfort indexes.

2. MATERIAL AND METHODS

Environmental conditions

The experiment was carried out in four thermal environment-controlled wind tunnels equipped with heating and air moistening function, housed in an experimental room with an area of 31.92 m². This room was equipped with two air conditioning systems with a power of 5,275 kW each, which were used to maintain the temperature inside the room below the target value.

All experimental procedures involving animals were previously approved by the Ethics Committee on Animal Use of the Federal University of Lavras, under protocol N° 079/17.

Experimental unit and management

Ninety 28-week-old *Hyline* laying hens at peak production were used. Laying hens were kept under thermoneutral conditions for one week, with an air dry-bulb temperature (t_{db}) of 23.2 ± 0.1 °C and relative humidity (RH) of $60.5\% \pm 0.8\%$ (Curtis, 1983; Albright, 1990; Baêta; Souza, 2010). For each group of birds, 7 days were used for acclimatization in the animal environment laboratory (Piestun et al., 2013; Abdelqader, Al - Fataftah, 2014; Kodaira et al., 2015). The ninety laying hens, in peak laying, were divided into three flocks of thirty birds for better conditioning in the experimental laboratory.

After adaptation, the hens were subjected to a factorial combination of five air dry-bulb temperatures (t_{db} : 20, 24, 28, 32 and 36 °C), two relative humidity (RH: 40 and 60%) and three air velocities (V : 0.2, 0.7 and 1.4 ms^{-1}), totaling 30 treatments. Each hen was exposed to thermal challenge only once. During acclimatization and thermal challenge, the hens received water and feed *ad libitum*. The light program adopted was 16 hours of light (L) and 8 hours of darkness (D) and illuminance of 5 lux.

Experimental Protocol

Laying hens were thermally challenged for at least 180 minutes or until stabilization of cloacal temperature but not exceeding the maximum of 6 hours, including animals exposed to 36°C. This length was defined based on Yanagi Junior's (2002) study with laying hens exposed to acute stress (35°C), in which the time required for stabilization of cloacal temperature ranged from 106 to 185 minutes.

Measurements

Data collections for all physiological variables were performed every 10 minutes of two treatments simultaneously (two tunnels in operation, one bird per tunnel, in an experimental test). During each test (thermal challenge), respiratory rate (RR), cloacal temperature (t_{clo}), and surface temperature (t_{sur}) of laying hens were measured at 10-min intervals. RR was measured by counting respiratory movements for 15 seconds and then multiplying it by four to obtain the number of breaths per minute (Castilho et al., 2015; Garcia et al., 2015; Marchini et al., 2007). The t_{clo} was measured using a digital thermometer (PT100

model Gulterm 200, accuracy ± 0.2 °C). The t_{sur} was measured every 10 minutes using a thermographic camera (model Ti 55, Fluke, the accuracy of 0.05 °C).

Experimental Design

Hens were allotted to a 5 x 2 x 3 factorial completely randomized design with three replicates. Statistical analyses were performed using R Core Team software (2015).

Cluster Analysis

Cluster dendrograms were generated using the R package fast cluster (R Core Team, 2015). Elements within the same *cluster* were similar, whereas elements in different *clusters* are different from one another.

Ward's method was used to create groups. It is a hierarchical clustering method that uses the sum-of-squared-errors as the measure of similarity. This method tends to create groups of approximately equal sizes due to the minimization of the internal variation (Hair et al., 2005).

We used the Euclidean distance analysis, which assumes that all relevant relationships between objects can be expressed by a matrix containing a measure of dissimilarity or proximity between each pair of objects.

Euclidean distance is the geometric distance in multidimensional space, calculated by equation 1 (Cook, 2004).

$$d(x,y)=\sqrt{\sum_{i=1}^p(x_i-y_i)^2} \quad (1)$$

where x_i and y_i are the i -th input vector and mean of the i -th variable, respectively.

Simple linear regression models (SLRM) and multivariate models

Simple linear regression models and multivariate models were adjusted for the following the physiological variables: t_{clo} (°C), t_{sur} (°C) and RR (mov. min^{-1}); thermal comfort indexes (BGHI, THI, RHL), enthalpy (H) and environment variables: t_{db} (°C), RH (%) and V (ms^{-1}), where:

BGHI: Black globe-humidity index (Buffington et al., 1981)

THI: Temperature-humidity index (Thom, 1959)

RHL: Radiant Heat Load (Esmay, 1982)

H: Enthalpy (Albright, 1990)

Statistical Indices

The following statistical indices were used to measure the goodness-of-fit of each empirical model: mean error or bias (ME), mean absolute error (MAE), mean square error (MSE), root mean square error (RMSE), mean absolute percentage error (MAPE) and Nash-Sutcliffe coefficient (NSE) calculated by equations 2 to 7, respectively.

$$ME = BIAS = \frac{1}{n} \sum_{i=1}^n (P_i - O_i) \quad (2)$$

$$MAE = \frac{1}{n} \sum_{i=1}^n |P_i - O_i| \quad (3)$$

$$MSE = \frac{1}{n} \sum_{i=1}^n (P_i - O_i)^2 \quad (4)$$

$$RMSE = \sqrt{\frac{1}{n} \sum_{i=1}^n (P_i - O_i)^2} \quad (5)$$

$$MAPE = \frac{1}{n} \sum_{i=1}^n \left| \frac{O_i - P_i}{O_i} \right| \times 100 \quad (6)$$

$$NSE = 1 - \left[\frac{\sum_{i=1}^n (P_i - O_i)^2}{(O_i - \bar{O})^2} \right] \quad (7)$$

where,

P_i = *i*-th predicted values (%);

O_i = *i*-th observed values (%);

n = total number of samples;

\bar{O} = average of observed values (%).

Akaike's information criterion (AIC)

Akaike (1974) showed that the bias is asymptotically given by p , where p is the number of parameters to be estimated on the model, according to equation 8:

$$AIC = -2\log L(\theta) + 2(p) \quad (8)$$

The smaller the value of AIC, the better the fit. If the difference in AIC values (ΔAIC) between two models with different numbers of parameters is positive, model I (with smaller number of parameters) is better; if contrary, model II is better. AIC test is not based on the traditional statistical hypothesis (F test) but allows determining which model is the most accurate (Floriano et al., 2006).

3. RESULTS

The values of thermal comfort indexes and enthalpy indicate a transition from a thermoneutral environment to a condition of heat stress. Under these circumstances, laying hens may have to adjust their metabolism, as evidenced by the interaction between t_{db} , RH, and V, as shown in Table 1.

Figures 1 and 2 illustrate the clustering process in which treatments were classified based on similarity or distance. Environmental-related variables (Figure 1) and thermal comfort indexes were assigned to each parameter - t_{db} , RH, and V (Figure 2).

Table 2 shows the multivariate and straightforward models adjusted for cloacal temperature (t_{clo} , °C). The best statistical indices were found for Equation 9. However, the remaining equations may be valid when using other variables for managing or controlling the thermal environment.

Tables 3 and 4 show the adjusted empirical models with their respective statistical indices for t_{sur} and RR as a function of environment variables, thermal comfort indexes, enthalpy, or physiological response of the laying hen.

Figure 3 (graphs A, B, and C) illustrates the thermoneutral zones for laying hens based on the environmental variables t_{db} , RH and V. New comfort zones based on t_{db} were found by using empirical models. No models were adjusted for RH and V because their values were fixed in the experimental design. In this study, the thermoneutral zones for t_{db} are illustrated in Figure 3A: [25.9; 29.9] based on RR and [22.2; 30.2] based on t_{sur} .

Figures 4 (graphs A and B) and 5 (graphs A and B) illustrate the thermoneutral zones for laying hens based on thermal comfort indices (THI, BGHI, and RHL) and enthalpy (H). Empirical models were adjusted for these variables and new comfort zones were found, corroborating with the literature.

The thermoneutral zones found in this study based on THI and BGHI are illustrated in Figures 4A and 4B, respectively. The values found in this study for THI were [67; 75] based

on RR and [68; 78] based on t_{sur} . For BGHI, the observed values were [68; 73] based on RR and [68; 79] based on t_{sur} .

The thermoneutral zones found in this study based on H and RHL are illustrated in Figures 5A and 5B, respectively. The values found in this study for H were [45; 56] based on RR and [43; 71] based on t_{sur} . For RHL, the observed values were [441.7; 465.6] based on RR and [441.2; 492.8] based on t_{sur} .

The thermoneutral zones found in this study based on t_{clo} , t_{sur} , and RR are illustrated in Figures 6A, 6B and 6C, respectively. For t_{clo} , the lower and upper critical limits were [39.4; 39.9] based on RR and [38.9; 40.6] based on t_{sur} , respectively. Thermal comfort limits for t_{sur} based on RR were [26.5; 29.9], whereas the thermal comfort limit for RR based on t_{sur} was [30; 67].

4. DISCUSSION

Values of BGHI ranging from 69 to 77 does not affect the performance of laying hens at peak production, indicating thermal comfort (Teixeira, 1991; Armstrong, 1994; Medeiros et al., 2005; Botelho et al., 2016). Therefore, values of BGHI within the thermoneutral zone (69 to 77) were also obtained in this study for hens exposed to temperatures of 24°C/40% RH and 28°C/60% RH, with wind speeds of 0.2; 0.7 and 1.4 ms^{-1} . Based on the findings of Tinôco (1998), in which the upper limit for laying hen comfort was established as a BGHI of 75, birds exposed to treatments from 20°C to 28°C and 40% RH would be in thermal comfort. BGHI values between 74 and 79 are considered safe, according to several studies (Silva et al., 2013; Baêta and Souza, 2010; Jácome et al., 2007).

Based on the findings of Barbosa Filho (2004) for laying hens, a THI from 71 to 75 is considered normal, 75 to 84 is danger status, and a THI from 84 to 87 is an emergency. This classification agrees with the findings of Jácome (2009) and Takahashi et al. (2009), who stated that THI values between 70 and 78 are considered comfortable for domestic animals, whereas values above 78 are considered stressful. In the present study, the treatments from 24°C and 60% RH to 28°C and 40% RH were within the thermoneutral zone. Temperatures above 28°C characterize heat stress (emergency). Physiological changes that directly affect the performance and egg quality are initiated in order to maintain homeothermy. Usually birds exposed to heat stress conditions with high air relative humidity increase their respiratory rate to dissipate excess heat from the airways to the environment (Oliveira et al., 2006).

The RHL is a mechanism of heat transfer from the surroundings to the bird. Rosa (1984) observed a mean RHL of 514.4 W m^{-2} in sheds with asbestos cement roof on a hot day ($t_{db} = 36^\circ\text{C}$). In this study, the values of RHL ranged from 426.6 to 531.7 W m^{-2} , indicating increased RHL compared with asbestos roofing, which is generally characterized by its low thermal comfort.

Studies on thermal comfort indices (RHL and BGHI) inside poultry facilities reported values outside the comfort limits for both broilers and laying hens. It reveals flaws in planning associated with a lack of a microclimate cooling system and items such as fans, sprinklers, and adiabatic cooling systems (Carvalho et al., 2012; Silva et al., 2013). Jácome et al. (2007) found RHL values (460.1 to 481.5 W m^{-2} with BGHI above 75) in poultry houses without acclimatization in northeastern Brazil, especially at the most critical times of the day. Bueno et al. (2018) highlighted that buildings with high ceilings provide more ventilation and, consequently, lower RHL values (478.01 W m^{-2}) than facilities with low ceilings, which have higher RHL values (516.66 W m^{-2}).

Silva et al. (2006) studied *Hy Line* laying hens placed in climate chambers and reported that the means for the amount of energy contained in dry air within the comfort limits based on enthalpy ranged from 64 to $70 \text{ kJ kg}_{\text{dry air}}^{-1}$. Vieira et al. (2010) developed a specific table for laying hens and established that the comfort zone for laying hens ranges from 58 and $68.8 \text{ kJ kg}_{\text{dry air}}^{-1}$. Barbosa Filho (2004) states that the comfort zone for laying hens based on enthalpy is between 71 and $75 \text{ kJ kg}_{\text{dry air}}^{-1}$ while in a stressful environment, it ranges from 84 and $87 \text{ kJ kg}_{\text{dry air}}^{-1}$. The results of the present study are consistent with the findings of Vieira et al. (2010) and Silva et al. (2006). At temperatures of 20°C and 40% and 60% RH, 24° and 40% and 60% RH and 28°C and 40% RH, the enthalpy was 38, 45, 45, 55 and $55 \text{ kJ kg}_{\text{dry air}}^{-1}$, below the thermal comfort accepted levels (between 64 and $70 \text{ kJ kg}_{\text{dry air}}^{-1}$).

Values of enthalpy within the thermoneutral zone were found for treatments 16-21, with t_{db} ranging from 28 to 32°C (Table 1) and H ranging from 66 and $68 \text{ kJ kg}_{\text{dry air}}^{-1}$. Under thermal stress conditions, the enthalpy ranges from 77 to $98 \text{ kJ kg}_{\text{dry air}}^{-1}$ at $32^\circ\text{C}/40\%$ RH and $36^\circ\text{C}/40$ and 60% RH.

The configuration of each dendrogram in relation to the number of clusters was defined according to the behavior of the physiological responses, thus contrasting, with each variable evaluated, so that there was a practical and coherent sense with the experimental scenarios evaluated.

Figure 1 shows that the response of environmental-related variables (t_{db} , RH, and V) was similar. Therefore, the treatments are grouped within the same cluster, where the

minimum, median and maximum values of temperature, relative humidity and wind speed are classified in the same range. This proximity between variables within the same cluster is associated with Euclidean Distance. Therefore, treatments that are close to each other are assigned to the same cluster.

In Figure 2, the responses of BGHI and THI indices are similar, i.e., the first clusters are organized from minimum to maximum temperatures (20, 24, 28, 32, and 36°C), which is related to the thermal comfort and stress ranges for laying hens. High temperatures (28°C/60%, 32°C/40 and 60% and 36°C/40 and 60% RH) indicate higher values for BGHI and THI, classified as an emergency.

The enthalpy (H) showed a different response. Enthalpy encompasses the internal energy of the system and the energy stored in the surroundings, thus representing the total energy of a system. It includes not only the energy of the closed system but also the energy exchanged with its surroundings. By dividing enthalpy groups into 4 clusters, it can be seen that treatments with lower enthalpy (38, 45, 45, 55 and 55 kJ kg_{dry air}⁻¹ at 20°C/40 and 60% RH, 24°/40 and 60% RH and 28°C/40% RH, respectively) are grouped in the first two clusters. Therefore, the amount of energy exchanged with the system in these conditions is smaller due to the low level of activity of hens, i.e., heat exchange of the body with the environment. However, this is not the optimal condition for the health of laying hens. The third cluster encompasses high temperatures (treatments 25 to 30; 36°C/40 and 60% RH; H between 77 and 98 kJ kg_{dry air}⁻¹), in which energy exchange with the surroundings increases significantly. In this case, birds activate physiological mechanisms to dissipate excess body heat to the environment, demonstrating that birds are under thermal stress. The fourth cluster encompasses the treatments classified as the thermoneutral zone based on H (treatments 16 to 21; 28°C/60% to 32°C/40% RH; H from 66 to 68 kJ kg_{dry air}⁻¹). It also includes treatments that are close to the group of thermal stress (22 to 24; 32°C/60% RH; H values of 82 kJ kg_{dry air}⁻¹).

The RHL was similar for each cluster of temperature, in which each cluster had a single temperature. The first cluster was composed of extremely high-temperatures (25 to 30; temperature 36°C/40 and 60% RH; RHL ranging from 526.8 to 531.1 W m⁻²), while the second cluster was composed of treatments with minimum temperatures (1 to 6; 20°C/40 and 60% RH; RHL ranging from 426.6 to 437.0 W m⁻²). The third cluster was composed of high-temperature treatments (19 to 24; 32°C/40 and 60% RH; RHL ranging from 498.9 to 504.2 W m⁻²) and the fourth cluster was composed of treatments with median temperatures (7 to 18; temperatures of 24°C/40 and 60% RH and 28°C/40 and 60% RH; RHL ranging from 450.0 to 479.4 W m⁻²). Rosa (1984) and Sartor et al. (2000) state that RHL is dependent on solar

radiation and heat accumulation capacity (thermal conductivity) of each material. Thus, the RHL can be determined by the amount of sensible and latent heat that must be removed (cooling) or absorbed (heating) by the environment in order to provide the recommended conditions of thermal comfort within the buildings. It is inferred, therefore, that treatments with temperatures ranging from 20 to 28°C were within the thermoneutral zone based on RHL [426.6 to 479.4 W m⁻²], in which heat exchange with the environment is mainly sensible, with the aid of fans, evaporative cooling systems, wind speed (air pockets within the buildings). RHL values rise [498,9 to 531,1 W m⁻²] under conditions of high temperatures (36°C), which implies increased respiratory rate and latent heat of evaporation. Thus, full pressurization of the environment is essential.

Simple linear regression models for estimation of t_{clo} (Table 2) showed reasonable adjustment, with low MAE values (0.3472 to 0.3990), low percentage errors (0.86 to 0.99%), besides the other statistical indices, showing that the generated models are well adjusted. In order to improve the performance of empirical models, multivariate regression models were adjusted for t_{clo} , showing adequate adjustments according to the independent variables listed in each model generated. The low values of BIAS (mean error) and MSE (mean square error) indices are highlighted.

Table 3 lists the simple linear models generated for t_{sur} . The low values of BIAS, MAE, RMSE, MAPE, NSE statistical indices indicate a satisfactory adjustment. For MSE (mean square error), equations 26 and 27 did not show a satisfactory adjustment, since the optimal value is equal to zero. Each value of AIC (Akaike information criterion) indicates the best-calculated model, i.e. the test allows determining which model is the most accurate and to what extent. We highlight the values of the correlation coefficient (R) of equations 21, 22, 23, 24 and 25 (above 70% correlation with the variables that make up each model).

Table 4 lists the simple linear models adjusted for RR. The MSE (mean square error) indicates the amplitude and variability of this parameter (519.32 to 1,281.79) since RR is subjective and has different comfortable temperature ranges compared with thermal stress ranges. In contrast, the ME (mean error) = BIAS ranged from -0.02 to 0.32, close to 0, and the NSE index (Nash-Sutcliffe coefficient) ranged from -1.60 to 0.71. These values are close to the limits from 0 to 1 NSE, classifying the model as acceptable (Celeste; Chaves, 2014).

In highlight, the best models for t_{clo} , t_{sur} and RR presented correlation coefficients of 0.6043; 0.9314 and 0.8613, respectively. In tables 2, 3 and 4 these models are characterized as equations 9; 21; 28, respectively, composed only by the variable t_{db} , they are easy to collect

within the production facilities, as well as models that presented an adequate adjustment to be used in the field.

After analyzing the thermal environments tested and information from the literature, graphs were prepared based on the zones of thermal comfort and heat discomfort. By comparing these results, we inferred new thermoneutral zones for variables that characterize the thermal environment of laying hens.

Brossi et al. (2009) indicate that the increase of ambient temperature negatively affects the heat dissipation capacity and acid-base homeostasis, leading to a condition of respiratory alkalosis and low productivity. The optimal productivity depends on the thermal conditions of the housing system, which in turn is dependent on the combined effects of air temperature, relative humidity, incident solar radiation, and wind speed in which birds are exposed (Garcia et al., 2012).

In high-temperature environments (heat stress), increased respiratory rate is the first thermoregulatory mechanism to be activated. Thus, birds increase their respiratory rate to exchange heat with the environment. It interferes with several natural processes such as the elimination of CO₂, which leads to decreased availability of bicarbonates (HCO₃) for eggshell formation, compromising its development (Albino et al., 2014). In this condition, the t_{clo} and t_{sur} concomitantly rise as t_{db} and RH increase. Nääs et al. (2010) observed that featherless areas are highly correlated with ambient temperature and that birds that lose more sensible heat have temperatures close to thermoneutrality and, consequently, greater latent heat dissipation at high temperatures.

5. CONCLUSION

Respiratory rate was the most appropriate physiological response to establish ranges of thermoneutrality for laying hens, since its response precedes the response of other variables, making it the most prudent choice.

The thermoneutral zones characterizing homeostasis for laying hens based on RR are as follows: from 25.9 to 29.9°C for t_{db} , from 67 to 75 for THI, from 68 to 73 for BGHI, from 45 to 56 kJ kg⁻¹ dry air⁻¹ for H and 441.7 to 465.6 W for RHL. Comfort limits for physiological responses t_{clo} , t_{sur} , and RR found in this study are 39.4 to 39.9 °C, 26.5 to 29.9°C, and 30 to 67 mov. min⁻¹, respectively.

The method for obtaining lower and upper comfort limits can be improved by increasing the number of replicates for each thermal challenge, as well as by using

mathematical or computational models that allow obtaining more accurate statistical indices. The number of repetitions and the use of mathematical modeling to be worked on, may directly impact the amplitude of each limit to be established for each variable of interest.

Acknowledgements

The authors thank the Federal University of Lavras, CAPES, and CNPq for the support for this research.

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Table 1. Thermal comfort indices and physiological responses of laying hens under different thermal environments

Treat.	$t_{db\ i}^1$	$RH\ i^2$	$V\ i^3$	$THI\ i^4$	$BGHI\ i^5$	$H\ i^{6t}$	$RHL\ i^7$	$t_{clo\ i}^8$	$t_{sur\ i}^9$	$RR\ i^{10}$
I										
1			0.2				426.6	39.80	24.90	26
2	20	40	0.7	64	65	38	428.6	39.80	24.70	31
3			1.4				428.3	39.90	24.80	25
4			0.2				437.0	39.80	26.20	32
5	20	60	0.7	66	67	45	433.2	40.00	26.60	33
6			1.4				431.8	39.70	25.10	27
7			0.2				450.5	40.30	31.00	37
8	24	40	0.7	69	70	45	452.3	39.90	29.60	38
9			1.4				459.6	40.20	31.00	37
10			0.2				450.0	39.80	28.50	36
11	24	60	0.7	71	72	55	457.5	39.90	28.00	32
12			1.4				453.2	40.30	27.80	41
13			0.2				476.7	39.90	31.70	32
14	28	40	0.7	74	75	55	477.9	39.60	31.70	29
15			1.4				479.4	39.90	31.30	37
16			0.2				475.4	39.80	33.50	38
17	28	60	0.7	76	77	68	478.0	40.10	32.30	28
18			1.4				476.2	40.10	33.40	42
19			0.2				499.5	40.30	33.40	40
20	32	40	0.7	79	80	66	498.9	40.70	34.50	39
21			1.4				502.5	40.00	33.50	47
22			0.2				499.2	40.30	36.60	47
23	32	60	0.7	82	82	82	501.2	40.20	35.20	68
24			1.4				504.2	39.80	36.30	42
25			0.2				528.1	40.80	36.90	117
26	36	40	0.7	84	85	77	527.7	41.10	36.20	122
27			1.4				531.1	40.50	37.20	132
28			0.2				526.8	40.60	35.80	163
29	36	60	0.7	87	88	98	530.7	40.60	36.90	145
30			1.4				530.7	41.20	36.90	145

¹ t_{db} : air dry-bulb temperature in °C; ²RH: air relative humidity in %; ³V: Air velocity, in m s⁻¹; ⁴THI: temperature-humidity index; ⁵BGHI: black globe-humidity index; ⁶H: enthalpy according to Albright (1990), in kJ kg⁻¹ dry air; ⁷RHL: radiant heat load in W m⁻²; ⁸ t_{clo} : cloacal temperature in °C; ⁹ t_{sur} : surface temperature in °C; ¹⁰RR: Respiratory rate, in mov. min⁻¹.

Table 2. Simple and multivariate linear regression models adjusted for cloacal temperature

Simple linear regression models for estimation of $t_{cloacal}$											
Equations	Equation #	F TEST ¹	ME = BIAS ²	MAE ³	MSE ⁴	RMSE ⁵	MAPE ⁶	R ⁷	NSE ⁸	AIC ⁹	Error (%)
$t_{clo} = 43.000238 - 0.268722 t_{db} + 0.005739 t_{db}^2$	09	2.67×10^{-9}	-0.0006	0.3518	0.1953	0.4419	0.88	0.6043	-0.7379	-140.82	0.88
$t_{clo} = 37.184 + 0.0395 \text{ THI}$	10	9.30×10^{-8}	-0.0016	0.3777	0.2220	0.4712	0.93	0.5275	-1.5940	-131.31	0.93
$t_{clo} = 37.1912 + 0.039 \text{ BGHI}$	11	1.25×10^{-7}	0.0024	0.3788	0.2235	0.4727	0.94	0.5229	-1.6499	-130.71	0.94
$t_{clo} = 36.258343 + 0.008151 \text{ RHL}$	12	1.25×10^{-7}	-0.0003	0.3744	0.2235	0.4728	0.93	0.5229	-1.6542	-130.72	0.93
$t_{clo} = 39.18751 + 0.015442 \text{ H}$	13	5.74×10^{-7}	-0.0005	0.3813	0.2310	0.4807	0.95	0.4989	-2.0188	-127.69	0.95
$t_{clo} = 38.34611 + 0.008151 t_{sur}$	14	1.40×10^{-5}	-0.0007	0.3990	0.2479	0.4979	0.99	0.4407	-3.1498	-121.39	0.99
$t_{clo} = 39.738343 + 0.007389 \text{ RR}$	15	5.52×10^{-10}	-0.0006	0.3472	0.1978	0.4447	0.86	0.5976	-0.8008	-141.55	0.86
Multivariate models for estimation of $t_{cloacal}$											
$t_{clo} = 39.421744 + 0.093035 t_{db} - 0.001071 \text{ RH} + 0.005783 \text{ V} - 0.057359 t_{sur}$	16	1.46×10^{-6}	-0.0005	0.3629	0.2106	0.4589	0.90	0.5615	-1.1679	-130.08	0.90
$t_{clo} = 36.78065 + 0.06450 \times \text{THI} - 0.04662 t_{sur}$	17	2.37×10^{-7}	-0.0008	0.3647	0.2166	0.4654	0.91	0.5440	-1.3775	-131.54	0.91
$t_{clo} = 36.82762 + 0.06085 \text{ BGHI} - 0.04112 t_{sur}$	18	3.99×10^{-7}	-0.0004	0.3689	0.2192	0.4682	0.92	0.5361	-1.4766	-130.46	0.92
$t_{clo} = 39.096050 + 0.014415 \text{ H} + 0.004884 t_{sur}$	19	3.93×10^{-6}	-0.0005	0.3827	0.2309	0.4806	0.95	0.4992	-2.0127	-125.73	0.95
$t_{clo} = 35.565541 + 0.011777 \text{ RHL} - 0.032894 t_{sur}$	20	5.07×10^{-7}	-0.0006	0.3684	0.2204	0.4695	0.92	0.5323	-1.5252	-129.97	0.92

¹ F test ($p < 0.05$); ² ME = BIAS: mean error; ³ MAE: mean absolute error; ⁴ MSE: mean square error; ⁵ RMSE: root mean square error; ⁶ MAPE: mean absolute percentage error; ⁷ R: correlation coefficient; ⁸ NSE: Nash-Sutcliffe coefficient; ⁹ AIC: Akaike's information criterion.

t_{clo} : cloacal temperature (°C); t_{db} : air dry-bulb temperature (°C), THI: temperature-humidity index, BGHI: black globe-humidity index, RHL: radiant heat load (W m^{-2}), H: enthalpy ($\text{kJ kg}^{-1}_{\text{dry air}}$); RR: respiratory rate (mov. min.₋₁); RH: relative humidity (%) and t_{sur} : surface temperature of the bird (°C).

Table 3. Simple and multivariate linear regression models adjusted for surface temperature

Equations	Equation #	F TEST ¹	ME = BIAS ²	MAE ³	MSE ⁴	RMSE ⁵	MAPE ⁶	R ⁷	NSE ⁸	AIC ⁹	Error (%)
$t_{sur} = 11.9998278 + 0.703792 t_{db}$	21	$<2.2 \times 10^{-16}$	0.0002	1.3523	2.4196	1.5555	4.37	0.9314	0.9723	83.52	4.37
$t_{sur} = -8.651 + 0.536 \text{ THI}$	22	$<2.2 \times 10^{-16}$	0.0007	1.3436	2.5105	1.5844	4.37	0.9288	0.9712	86.86	4.37
$t_{sur} = -8.8428 + 0.5323 \text{ BGHI}$	23	$<2.2 \times 10^{-16}$	0.0011	1.3512	2.5586	1.5996	4.38	0.9273	0.9707	88.57	4.38
$t_{sur} = -21.0618 + 0.1103 \text{ RHL}$	24	$<2.2 \times 10^{-16}$	0.0225	1.4339	2.8637	1.6922	4.62	0.9182	0.9669	98.68	4.62
$t_{sur} = 18.7281 + 0.2059 \text{ H}$	25	$<2.2 \times 10^{-16}$	-0.0028	1.8524	4.6162	2.1485	6.05	0.8645	0.9458	141.69	6.05
$t_{sur} = -104.504 + 3.392 t_{clo}$	26	1.40×10^{-5}	0.0132	3.2591	14.7210	3.8368	10.85	0.4407	0.8032	246.07	10.85
$t_{sur} = 28.282 + 0.0601 \text{ RR}$	27	2.89×10^{-11}	-0.0001	2.7183	11.0438	3.3232	9.10	0.6289	0.8598	219.97	9.10

¹ F test ($p < 0.05$); ² ME = BIAS: mean error; ³ MAE: mean absolute error; ⁴ MSE: mean square error; ⁵ RMSE: root mean square error; ⁶ MAPE: mean absolute percentage error; ⁷ R: correlation coefficient; ⁸ NSE: Nash-Sutcliffe coefficient; ⁹ AIC: Akaike's information criterion.

t_{sur} : surface temperature of the bird (°C); t_{db} : air dry-bulb temperature (°C), THI: temperature-humidity index, BGHI: Black globe-humidity index, RHL: radiant heat load (W m^{-2}), H: enthalpy ($\text{kJ kg}^{-1} \text{ dry air}$), t_{clo} : cloacal temperature (°C) and RR: respiratory rate (mov. min^{-1}).

Table 4. Simple and multivariate linear regression models adjusted for respiratory rate

Equations	Equation #	F TEST ¹	ME = BIAS ²	MAE ³	MSE ⁴	RMSE ⁵	MAPE ⁶	R ⁷	NSE ⁸	AIC ⁹	Error (%)
$RR = 501.366667 - 39.247222 t_{db} + 0.802083 t_{db}^2$	28	$<2.20 \times 10^{-16}$	0.0034	16.8427	519.3208	22.7886	34.59	0.8613	0.7068	568.40	34.59
$RR = -273.218 + 4.385 \text{ THI}$	29	6.56×10^{-16}	-0.0077	25.1400	956.8482	30.9330	53.82	0.7241	0.2838	621.30	53.82
$RR = -276.73 + 4.38 \text{ BGHI}$	30	4.28×10^{-16}	-0.0223	25.0506	947.6605	30.7841	53.83	0.7272	0.2952	620.43	53.83
$RR = -384.035 + 0.9214 \text{ RHL}$	31	2.41×10^{-16}	-0.0023	24.9435	934.7180	30.5732	53.17	0.7316	0.3115	619.27	53.17
$RR = -59.704 + 1.851 \text{ H}$	32	$<2.20 \times 10^{-16}$	-0.0168	22.5850	908.7127	30.1449	47.54	0.7404	0.3433	616.61	47.54
$RR = -1876.64 + 48.15 t_{clo}$	33	5.52×10^{-10}	0.3194	26.5933	1281.7955	35.8022	52.31	0.6023	-0.3058	648.83	52.31
$RR = -152.394 + 6.603 t_{sur}$	34	2.89×10^{-11}	0.0085	28.0427	1216.0019	34.8712	57.59	0.6289	-0.1276	642.90	57.59

¹ F test ($p < 0.05$); ² ME = BIAS: mean error; ³ MAE: mean absolute error; ⁴ MSE: mean square error; ⁵ RMSE: root mean square error; ⁶ MAPE: mean absolute percentage error; ⁷ R: correlation coefficient; ⁸ NSE: Nash-Sutcliffe coefficient; ⁹ AIC: Akaike's information criterion.

RR: respiratory rate (mov. min^{-1}), t_{db} : air dry-bulb temperature ($^{\circ}\text{C}$), THI: temperature-humidity index, BGHI: black globe-humidity index, RHL: radiant heat load (W m^{-2}), H: enthalpy ($\text{kJ kg}^{-1}_{\text{dry air}}$), t_{clo} : cloacal temperature ($^{\circ}\text{C}$) and t_{sur} : surface temperature of the bird ($^{\circ}\text{C}$).

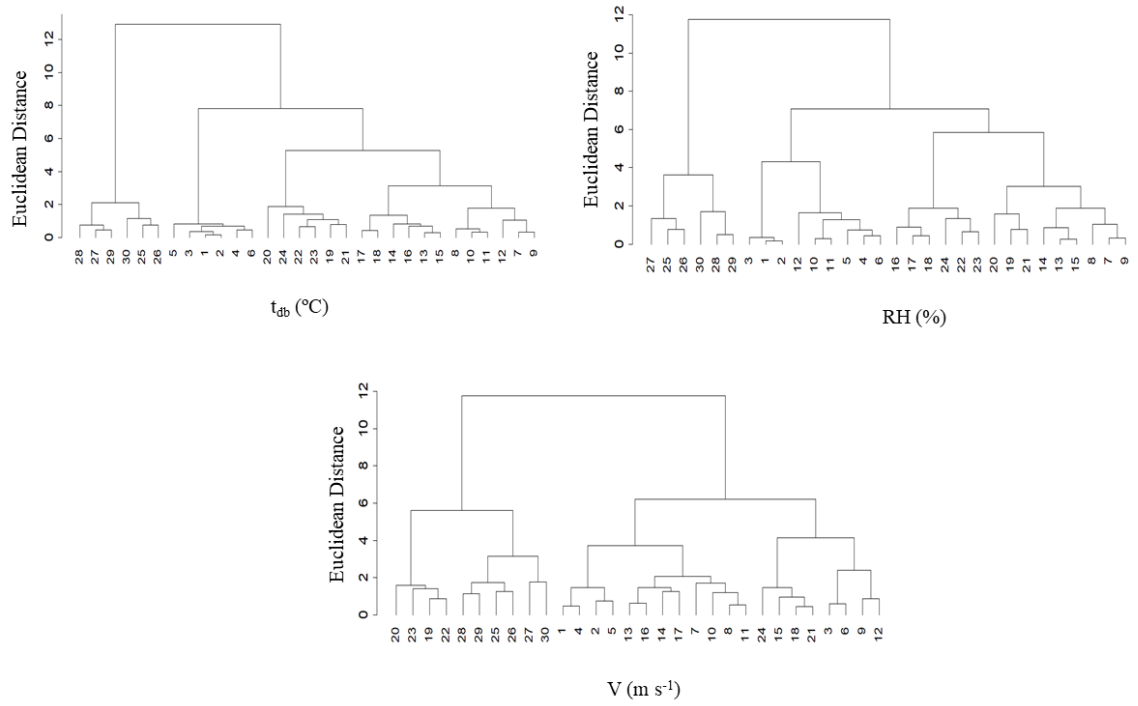


Figure 1. Clustering dendrograms of environment variables: air dry-bulb temperature, relative humidity and velocity (t_{db} , RH, and V, respectively).

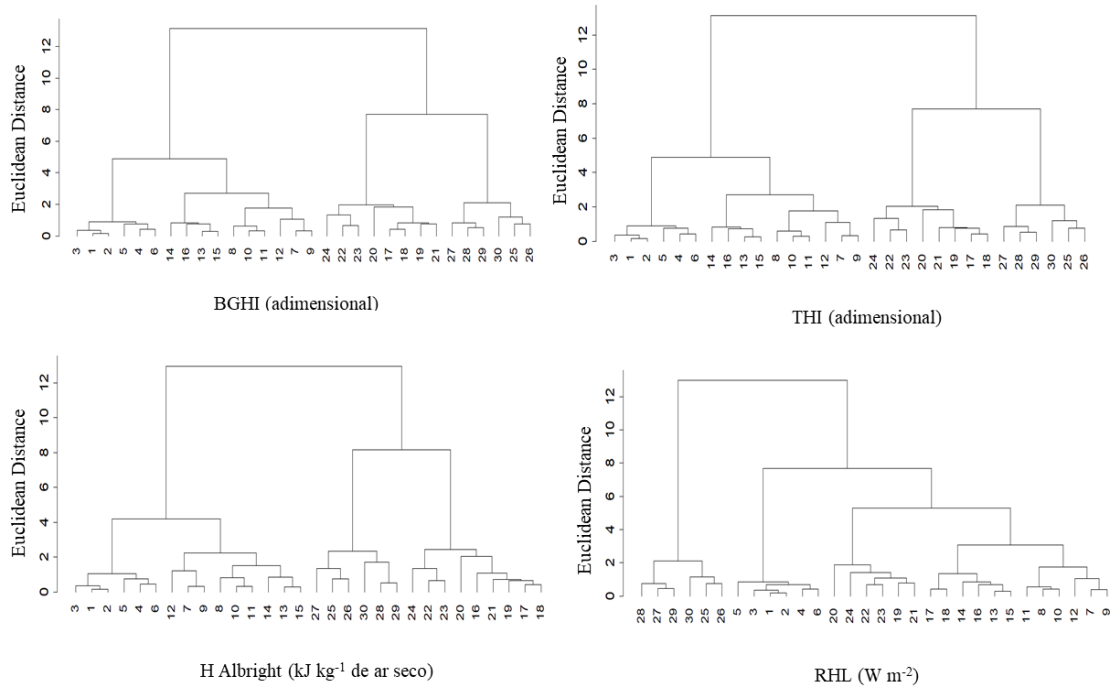


Figure 2. Clustering dendrograms of thermal comfort indices (BGHI: Black globe-humidity index, THI: temperature-humidity index and RHL: radiation heat load and H: enthalpy).

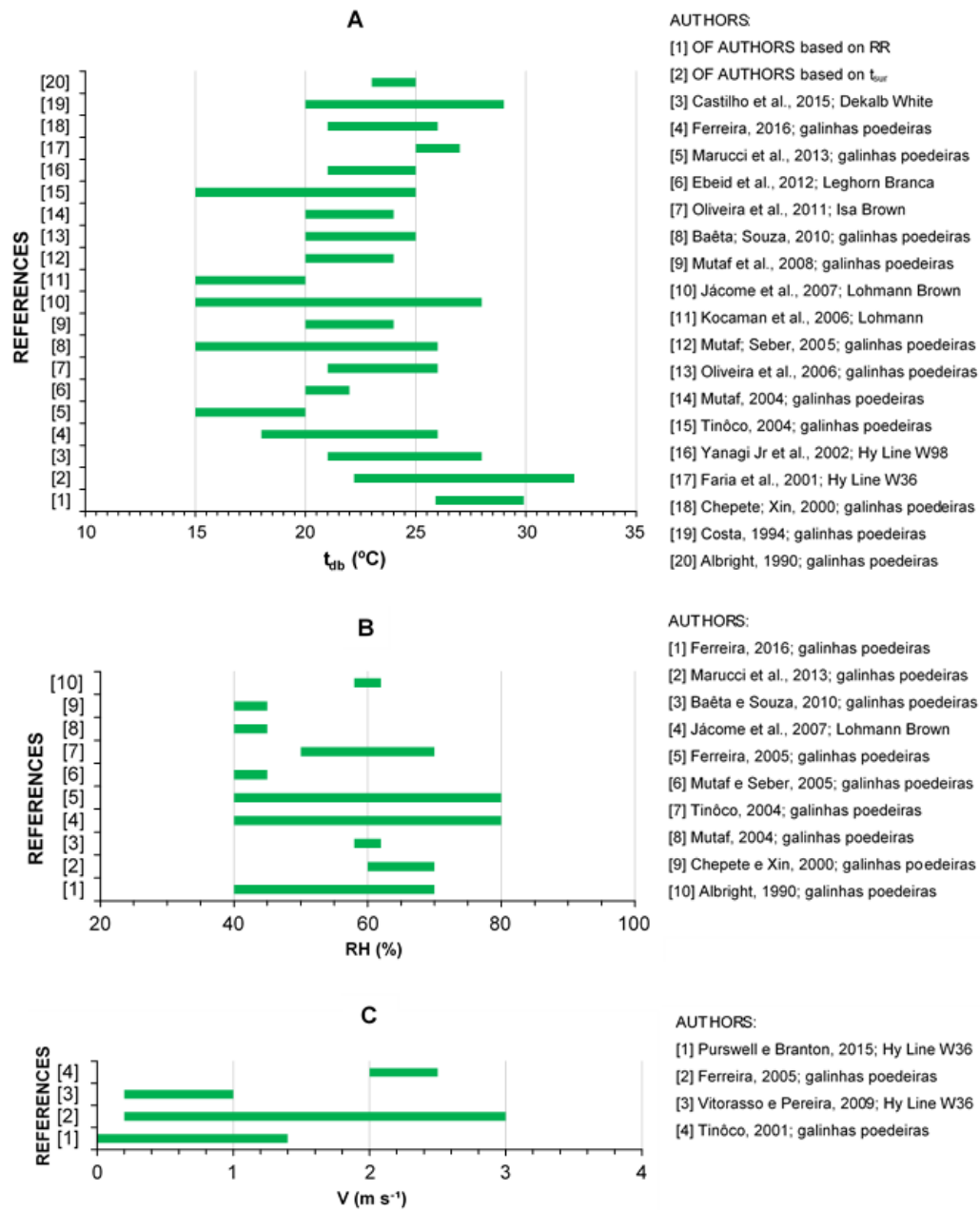


Figure 3. Graphs of thermoneutral zones based on environmental variables: air dry-bulb temperature (graph A), relative humidity (graph B) and velocity (graph C) (t_{db} , RH, and V, respectively).

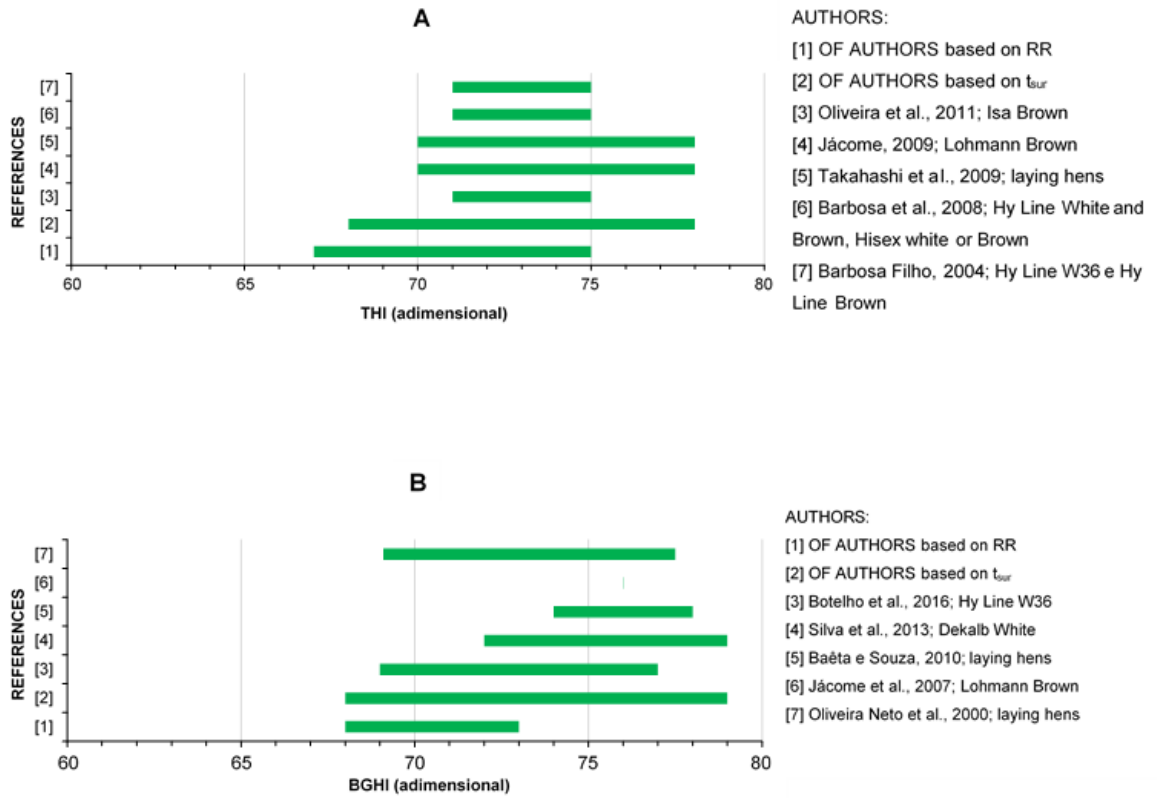


Figure 4. Graphs of thermoneutral zones based on thermal comfort indices: Temperature-Humidity Index (THI) (Graph A) and Black Globe-Humidity Index (BGHI) (Graph B).

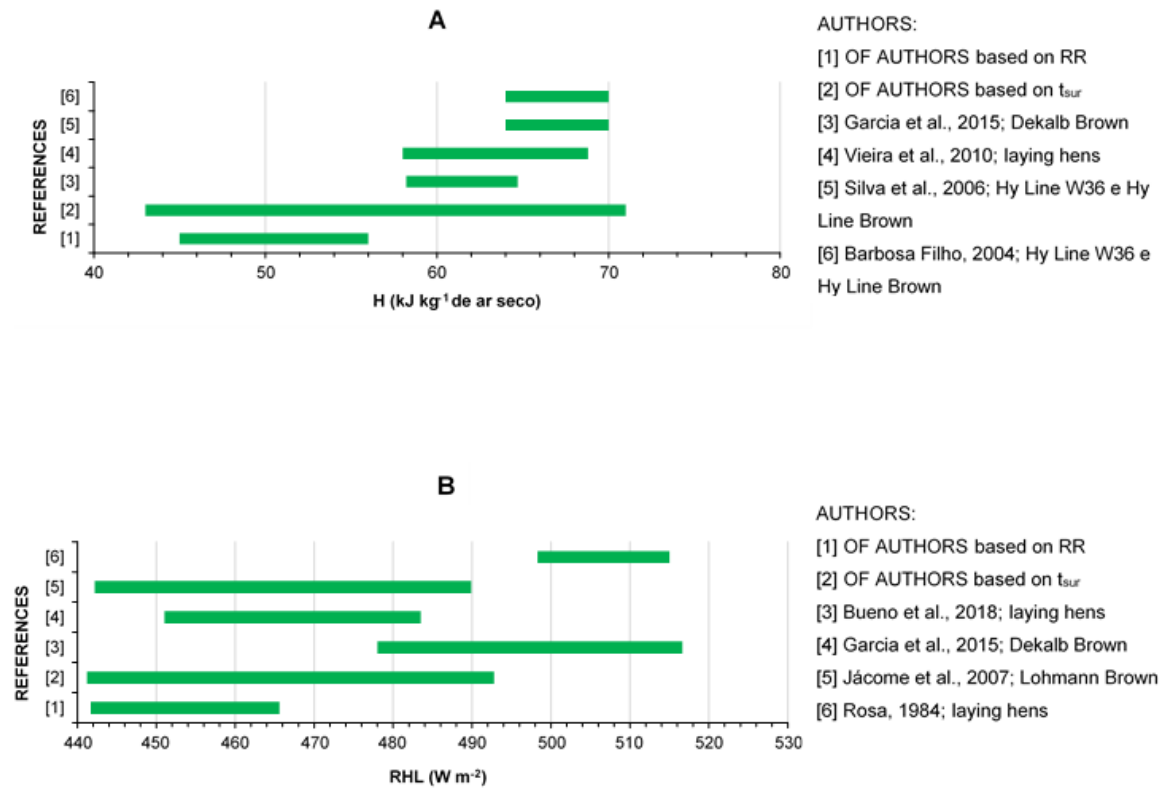


Figure 5. Graphs of thermoneutral zones based on enthalpy (H) (graph A) and radiant heat load (RHL) (graph B).

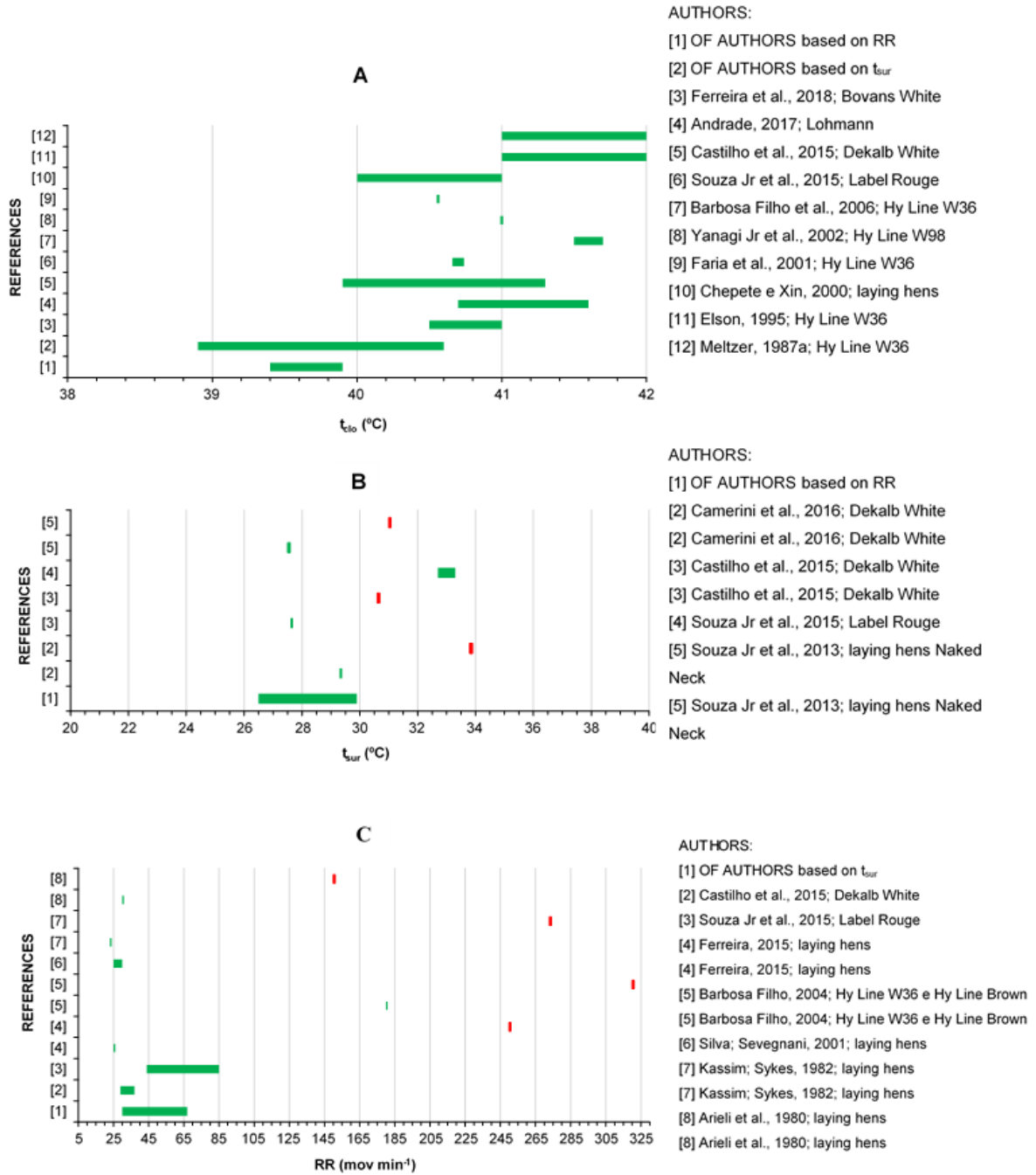


Figure 6. Graphs of thermal comfort ranges for physiological variables: cloacal temperature (t_{clo}) (graph A), surface temperature (t_{sur}) (graph B), respiratory rate (RR) (graph C).



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**ARTICLE 2 - PERFORMANCE OF EMPIRICAL AND ARTIFICIAL
INTELLIGENCE-BASED MODELS FOR THE PREDICTION OF CLOACAL
TEMPERATURE AND RESPIRATORY RATE OF LAYING HENS**

(DRAFT VERSION)

Article prepared for journal submission *Computers and Electronics in Agriculture*.

**PERFORMANCE OF EMPIRICAL AND ARTIFICIAL INTELLIGENCE-BASED
MODELS FOR THE PREDICTION OF CLOACAL TEMPERATURE AND
RESPIRATORY RATE OF LAYING HENS**

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ABSTRACT: The aim of the present work consisted in developing empirical and artificial intelligence-based models to predict thermal comfort and discomfort scenarios inside laying hens buildings using two physiological variables: cloacal temperature and respiratory rate. Empirical and artificial intelligence-based models were developed (fuzzy logic and artificial neural networks - ANN). The best models were composed of air dry-bulb temperature (t_{db}), relative humidity (RH) and air velocity (V) as input variables, while cloacal temperature ($t_{cloacal}$) and respiratory rate (RR) were the output variables. The artificial intelligence methodologies resulted in coefficients of determination of 0.9933 and 0.8002 for the fuzzy system and RNAs trained to predict RR (mov min^{-1}) and $t_{cloacal}$ ($^{\circ}\text{C}$) variables, respectively.

Keywords: thermal comfort; physiological variables; fuzzy logic; artificial neural networks; poultry farming.

1. INTRODUCTION

Physiological variables are used to assess thermal comfort in livestock. When exposed to variations in thermal comfort parameters, the animals initiate attempts to maintain thermal balance. In a scenario of high temperature-induced stress, the birds raise their body temperature and respiratory rate as a thermoregulatory mechanism. These physiological

processes are based on the increase of heat dissipation and decrease of metabolic heat production to maintain body homeothermy (YAHAV et al., 2005). The adequate room temperature for the thermal comfort of laying hens ranges between 21 and 28°C (CASTILHO et al., 2015). Ferreira (2016) claims that adult laying hens are more productive when raised in environments whose relative humidity ranges between 40% and 70%.

High temperatures in tropical countries may lead to significant concerns about animal productivity in poultry production systems. One of the limiting factors is their inability to dissipate heat in such high-temperature conditions since birds do not possess sweat glands and their bodies are covered in feathers (ALBINO et al., 2014).

In high temperature-induced stress conditions, birds increase their respiratory rate in up to ten times its regular rate in response to thermal discomfort, indicating the use of evaporation mechanisms of cooling (SANTOS et al., 2006). In high-temperature conditions associated with high air humidity, birds may develop respiratory alkalosis (BORGES et al., 2003).

The body temperature of birds increases when the room temperature quickly reaches 30°C (BOONE; HUGHES, 1971). When the air temperature increases gradually, the body temperature is relatively steady until the room temperature reaches 33°C (CAMERINI et al., 2016). Increased cloacal temperature is a physiological response to conditions of high temperature and relative humidity, resulting from the storage of metabolic heat (SILVA et al., 2003). For cloacal temperature, the thermal comfort may vary from 41 to 42°C (OLIVEIRA et al., 2006).

Attempts to solve thermal-related issues in livestock are based on the use of tools that maximize and optimize the monitoring of the thermal environment, namely artificial intelligence, which approaches human and computer decisions. Intelligent systems are capable of processing imprecise information and turn them into an easily computer-implemented mathematical language (FERREIRA et al., 2012). Among the most used artificial intelligence-based systems in the area of thermal comfort data analysis, we can highlight the Artificial Neural Networks (ANNs) (SILVA et al., 2010) and the fuzzy systems (ZADEH, 1965).

The ANNs are models that consist of a group of simple interconnected cells, called artificial neurons, organized in layers and which calculate mathematical functions (MATIN et al., 2012). They are fundamental conceptions to the functioning of the human brain, being, hence, capable of learning from experience (SILVA et al., 2010).

The fuzzy set theory is a mathematical theory applied to diffuse concepts whose reasoning is approximate, similar to human thought. The building process of the model is based on the steps of fuzzification (conversion of input values into fuzzy values), inference (determination of output values based on the pre-established rule systems), and defuzzification (conversion of fuzzy values into numeric values) (SILVA; BASSANEZI, 2006; TAVARES; SCHIASSI, 2016).

The present study aimed to develop conventional and artificial intelligence-based models to predict thermal comfort and discomfort scenarios inside laying hen buildings using the physiological variables cloacal temperature and respiratory rate.

2. MATERIAL AND METHODS

All experimental procedures involving animals were previously approved by the Ethics Committee on Animal Use (CEUA) of the Federal University of Lavras, under protocol N° 079/17.

Data were collected using four thermal environment-controlled wind tunnels equipped with heating and air moistening function, housed in an experimental room with an area of 31.92 m². This room was equipped with two air conditioning systems, which were used to control the temperature outside the wind tunnels and thus help the air temperature inside the tunnels to reach the target value. This room was equipped with two air conditioning systems, which were used to keep the temperature inside the room below the target value. Ninety laying hens of the *HyLine* strain, 28 weeks of age, were used in peak laying. The animals were subjected to a factorial combination of five air dry-bulb temperatures (t_{db} : 20, 24, 28, 32 and 36 °C), two air relative humidity (RH: 40 and 60%) and three air velocities (V: 0.2, 0.7 and 1.4 ms⁻¹), totaling 30 treatments. Each laying hen was exposed to thermal challenge only once. The accuracy of the sensors for measuring t_{db} , RH and V were 0.3 °C, 1 % and 0.1 m s⁻¹, respectively.

Birds were allowed seven days for adaptation to facilities before the thermal challenge (PIESTUN et al., 2013; ABDELQADER et al., 2014; KODAIRA et al., 2015). During the acclimatization period, the birds were subject to a room air temperature of 23.2±0.1°C and RH of 60.5%±0.8%, conditions of thermal comfort for them (CURTIS, 1983; ALBRIGHT, 1990; BAËTA; SOUZA, 2010), i.e. within the interval between 18 to 25°C for t_{db} and 40 to 60% for RH (MARUCCI et al., 2013; JÁCOME et al., 2007).

During each test, respiratory rate (RR) and cloacal temperature (t_{cloacal}) of birds were measured at 10-minute intervals until the stabilization of at least six readings. Laying hens were thermally challenged for at least 180 minutes or until stabilization of cloacal temperature but not exceeding the maximum of 360 minutes. This length was defined based on Yanagi Júnior et al. (2012) study with laying hens exposed to acute stress (35°C), in which the time required for stabilization of cloacal temperature ranged from 106 to 185 minutes. RR was measured by counting respiratory movements for 15 seconds and then multiplying it by four to obtain the number of breaths per minute (CASTILHO et al., 2015; GARCIA et al., 2015; MARCHINI et al., 2007). The measurement of t_{cloacal} was performed at 10 minute intervals using a digital thermometer with an accuracy of $\pm 0.2^\circ\text{C}$. Surface temperatures of the birds were also measured through an infrared camera with an accuracy of 0.05°C .

The birds were allotted to a 5 x 2 x 3 factorial completely randomized design (CRD) with three replicates. The analysis of variance and adjustment of empirical models were performed using R Core Team software (2015). Fuzzy logic and ANN models were developed using the software MATLAB® version R2011b (7.13.0.564).

2.1. Artificial intelligence

2.1.1. Artificial Neural Network (ANN)

The methodology proposed by Hernández-Julio et al. (2014) and Ponciano Ferraz et al. (2014) was used to develop ANN models. The developed ANN had two feed-forward layers, trained using the Levenberg-Marquardt backpropagation algorithm. The model settings included hidden layers, transfer functions of each hidden layer, the number of neurons, the learning rate, and the momentum, weight, and bias of the neurons.

Two subsets were randomly separated: training (70% of the data) and validation (30% of the data) (HERNANDEZ – JULIO et al., 2019; ABDEL-ZAHER, ELDEIB, 2016; BROWN-BRANDL et al., 2005). The first subset was used to obtain the optimal weights associated with the neurons (Figure 1). The validation set was used to reach the ideal number of hidden neurons or to determine an endpoint to the backpropagation algorithm. The number of hidden neurons varied according to each methodology. The configurations with the highest coefficients of determination (R^2) and the smallest mean square error (MSE) were selected (FERRAZ et al., 2014).

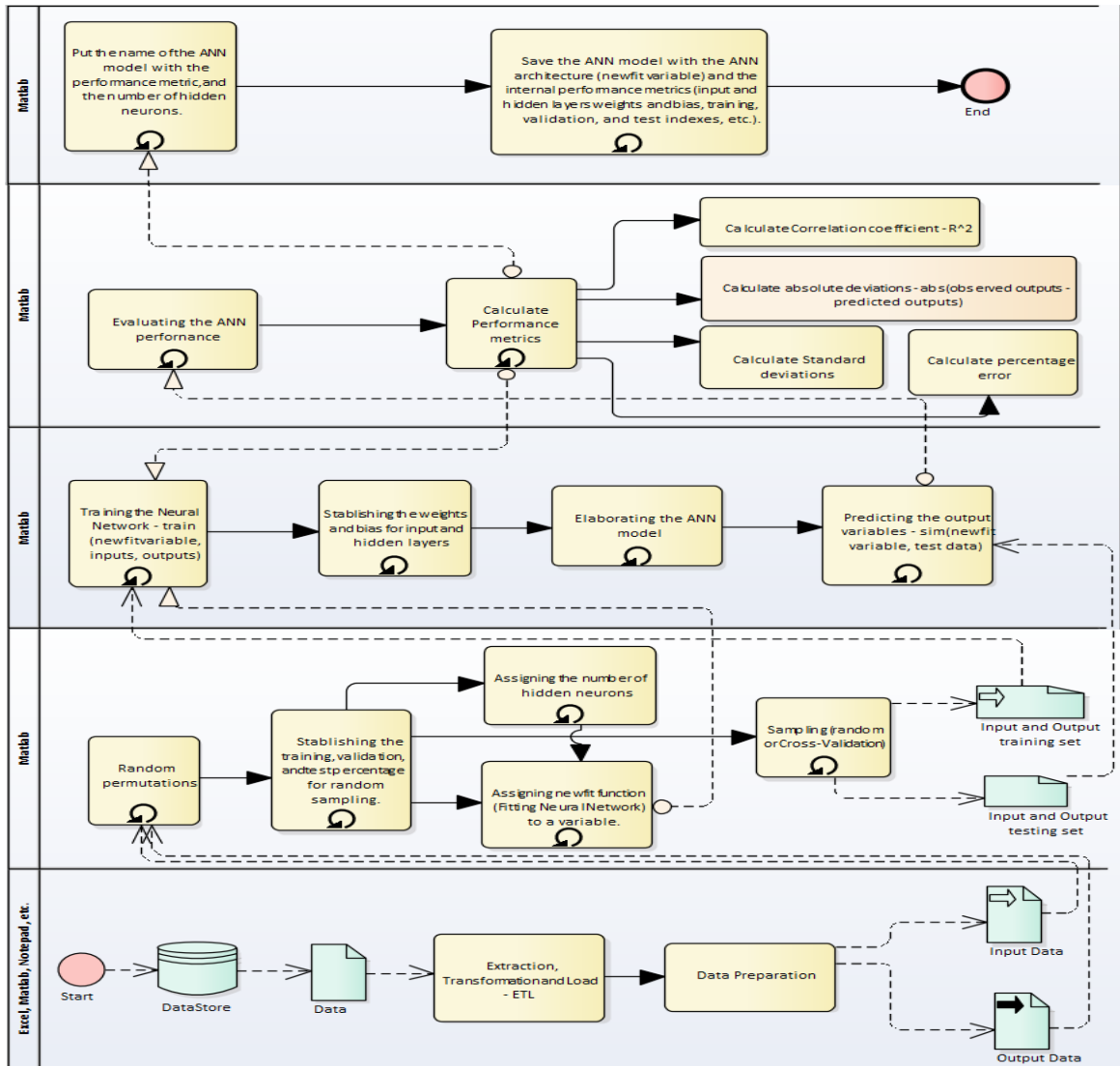


Figure 1. Flowchart showing the algorithm for the implementation of artificial neural networks (ANN) to predict laying hens cloacal temperature.

2.1.2. Fuzzy logic

The Mamdani inference method was used to develop the fuzzy systems. It has as an outcome a fuzzy set originated from the combination of input values and their respective pertinence degrees using the minimum operator, followed by the superposition of the rules with the use of the maximum operator (AMENDOLA, SOUZA, 2004). Defuzzification was carried out by applying the Center of Gravity method (Centroid or Center of Area), which considers all output possibilities, turning the fuzzy set originated by the inference into a numeric value (AMENDOLA, SOUZA, 2004).

The methodology used for the development of the fuzzy inference systems was based on the studies of Hernández-Julio et al. (2019), Hernández-Julio et al. (2018) and Hernández-

Julio et al. (2019). According to Hernández-Julio et al. (2019), the framework consists of the following steps:

1. **Dataset identification:** as mentioned in the previous section, the data used in this study were obtained under laboratory conditions. During the data collection process, all animals were treated following with the ethical standards of the Ethics Committee on Animal Use (CEUA) of the Federal University of Lavras, under protocol N° 079/17. The dataset consists of 90 instances and five attributes or variables (three input variables and two output variables). There are no missing values. Attributes were integer and real. The task associated with this problem is a regression.
2. **Data preparation (input):** data were organized in a Microsoft Excel spreadsheet. Three repetitions were obtained for each combination of input variables. In this step, the mean value was determined for each combination.
3. **Review of existing models:** the main objective of this step was to search for related studies and compare them to other results reported in the literature. Research on laying hens under thermal stress conditions was performed on the databases *Science Direct*, *Scopus*, *Google Scholar* and *Web of science*. The results will be presented in the Results and Discussion section.
4. **Determination of the optimal number of clusters:** dynamic tables were applied for each input variable (3). Most of the input variables were larger than 20 fuzzy sets; hence, the recommendation made by the author of the framework was followed (HERNÁNDEZ – JULIO et al., 2019). The rounded square root of every input variable higher than 20 was determined. Table 1 shows the optimal number of clusters for each input and output variable.

Table 1. The optimal number of clusters for each input and output variable

	Input variables			Output variables	
	Air dry-bulb temperature (°C)	Relative humidity (%)	Air velocity (m s ⁻¹)	Cloacal temperature (°C)	Respiratory rate (mov min ⁻¹)
Number of lines	72	68	3	64	53
Square root (rounded)	8	8	3	8	7

5. **Defining the minimum and the maximum number of clusters:** for this case study, the minimum number of groups was two.
6. **Random permutation:** the input and output data were randomized and permuted. Subsequently, the data were processed using the Levenberg–Marquardt back propagation algorithm.
7. **Fuzzy process:** for this case study, we applied the clustering method. Ward's method was used to classify each input and output variable. We selected the Euclidian distance as a distance measurement. As stated in Hernández-Julio et al. (2019), the fuzzy knowledge base is obtained in this step.
8. **Sampling:** for this case study, the dataset was trained using the random sampling method. The percentages for the case study were 70% for training and the remaining 30% for validation. In this stage, the extraction of the rule base was obtained using dynamic tables.
9. **Dynamic tables:** for this case study, the command “unique” was applied for the development of the following substeps:
 - 9.1. **Combining clusters datasets:** the combination of the cluster datasets was performed using the commands “nchoosek” and “unique” for matrices.
 - 9.2. **Establishing the fuzzy rules:** step based on the previous one. It processes the rule base of the fuzzy system. It was performed by following the recommendations in the previous section (with the command “unique”).
10. **Elaborating the Decision Support System based on the fuzzy set theory:** the data-oriented fuzzy systems were implemented on the Matlab[®] platform (The Mathworks Inc., R2011b). The following components were used: definition of linguistic variables, the set of rules, the number of sets, and the values of the Membership function for each variable. We used the proposed algorithms mentioned in the literature (HERNÁNDEZ – JULIO et al., 2019). The recommendation for this stage is that the modeler manually adjusts the set values suggested by the methodology until they reach the target values. The data were analyzed by researchers with more than ten years of experience in this research area, having been subject to a selection methodology proposed (CORNELISSEN et al., 2002) and used by several authors (YANAGI JÚNIOR et al., 2012; SCHIASSI et al., 2014). Following the combination, the output datasets were transformed into precise system output.
11. **Evaluating the performance of fuzzy inference systems:** in this case, for regression problems, the following statistics indices were used in the calculations of each output

variable: mean absolute error (MAE), root mean square error (RMSE), standard deviation (SD) and mean absolute percentage error (MAPE).

2.2. Simple linear regression models (SLRM) and multivariate models

Simple linear regression models and multivariate models were adjusted for the following physiological variables: $t_{cloacal}$ (°C) and RR (mov. min^{-1}); thermal comfort indexes (BGHI, THI, RHL), enthalpy (H) and environment variables: t_{db} (°C), RH (%) and V (m s^{-1}), where:

BGHI: Black globe-humidity index (BUFFINGTON et al., 1981);

THI: Temperature-humidity index (THOM, 1959);

RHL: Radiant Heat Load (ESMAY, 1982);

H: Enthalpy (ALBRIGHT, 1990).

2.3. Statistical Indices

The following statistical indices were used to measure the goodness-of-fit of each conventional model: mean absolute error (MAE), mean square error (MSE), root mean square error (RMSE) and mean absolute percentage error (MAPE) (equations 1, 2, 3 e 4, respectively).

$$\text{MAE} = \frac{1}{n} \sum_{i=1}^n |P_i - O_i| \quad (1)$$

$$\text{MSE} = \frac{1}{n} \sum_{i=1}^n (P_i - O_i)^2 \quad (2)$$

$$\text{RMSE} = \sqrt{\frac{1}{n} \sum_{i=1}^n (P_i - O_i)^2} \quad (3)$$

$$\text{MAPE} = \frac{1}{n} \sum_{i=1}^n \left| \frac{O_i - P_i}{O_i} \right| \times 100 \quad (4)$$

where,

P_i = *i*-th predicted values (%);

O_i = *i*-th observed values (%);

n = total number of samples.

3. RESULTS AND DISCUSSION

Tables 2 and 3 show the list of simple and multivariate models with their statistical indices for the physiological variables: cloacal temperature ($^{\circ}\text{C}$) and respiratory rate (mov min^{-1}), respectively, as a function of the environmental conditions, enthalpy, and thermal comfort indices.

Empirical models adjusted to t_{cloacal} with two or three variables (multivariate model) were not significant by F-test (Fisher) ($p > 0.05$) (equations 1 to 8; Table 2). On the other hand, models with only one variable were significant by F-test ($p < 0.05$).

Also in Table 2, we can observe significant improvement in all developed models (1 to 8) when two other methodologies for data analysis are applied – ANN and fuzzy logic: all the statistic indices had adequate adjustment, i.e., the systematics of calculation and structuring of data of each methodology grants a distinct array to the dataset. For t_{cloacal} , in particular, the ANN methodology showed the best adjustment compared with fuzzy and conventional models, showing the best statistical indices for both methodologies, especially for model number 1.

For t_{cloacal} , the best adjustment model (model 1, table 2) correlates variables that characterize the thermal environment (t_{db} , RH, V) with the physiological response of birds in the face of distinct thermal scenarios. In heat-induced stress scenarios, as the air temperature rises, the cloacal temperature also increases, i.e., the bird activates physiological mechanisms when facing high temperature and relative humidity conditions, resulting from the storage of metabolic heat (SILVA et al., 2003). Thermal stress is usually accompanied by reduced feed intake with a consequent reduction in productivity and egg production, production of small eggs, soft-shelled eggs and the occurrence of cloacal prolapse.

For t_{cloacal} , the ANN developed adopted the *multilayer-perceptron* (MLP) architecture, composed of three layers: input layer containing three neurons, hidden layer composed of 185 neurons and an output layer with one neuron (Figure 2). Two feedforward layers and supervised training were employed with the Levenberg-Marquardt (LM) backpropagation training algorithm, considered to be the quickest method to train such networks (BARBOSA et al., 2005; SILVA et al., 2012).

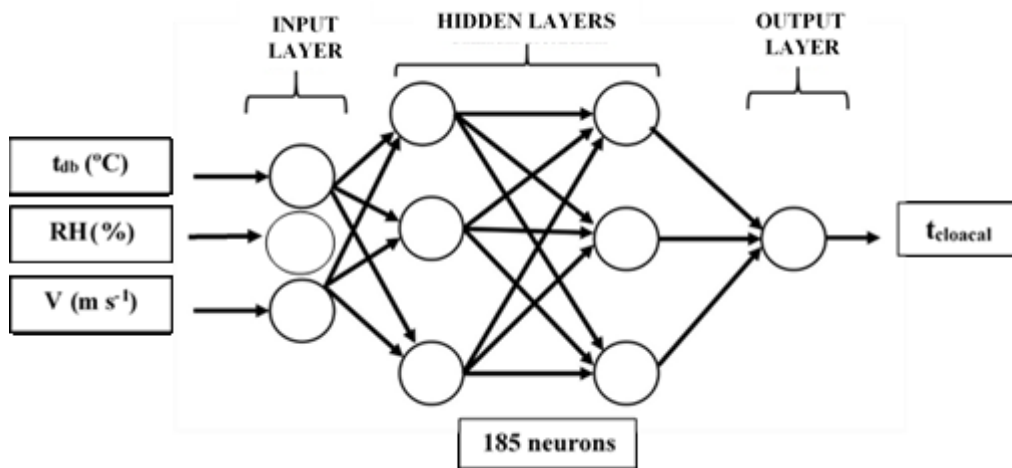


Figure 2. Configuration of the ANN for cloacal temperature ($t_{cloacal}$).

In Table 3, the empirical models developed for RR with two or three variables do not present statistical significance when submitted to the F-test ($p > 0.05$) (equations 1 to 8). The simple empirical models presented statistical significance ($p < 0.05$) and were better fitted compared with models adjusted to $t_{cloacal}$. Similarly, the methodologies based on artificial intelligence (fuzzy and ANN) were better fitted to all models compared with empirical models, and the fuzzy logic showed the best fit to the model number 1.

Based on the model with best fit for RR (model 16, table 3), which is composed of t_{db} , RH and V , we can infer that birds increase their respiratory rate in up to ten times the regular rate under heat-induced stress conditions as a response to the thermal discomfort, which indicates the use of evaporation mechanisms in the heat transfer (SANTOS et al., 2006). In high-temperature conditions associated with elevated air relative humidity, birds can develop respiratory alkalosis (BORGES et al., 2003). Overall, heat loss in birds is controlled by changes in blood flow on the body surface, or by altering the evaporation rate in the respiratory tract (ABREU et al., 2007).

By using the models developed for $t_{cloacal}$ and RR, we can observe the interaction between the thermal environment and physiological responses of birds. This interaction is due to the thermoregulation process of animals in high-temperature conditions, which include the interaction of physiology through heat-dissipating mechanisms.

Birds under thermal stress face difficulties in reaching thermal balance with the environment. It is due to the absence of sweat glands whose function is to dissipate the excess of body heat through the evaporation of sweat. According to Zhao et al. (2013), the thick layer of feathers covering almost all of the body surface acts as a resistance factor to heat loss. Therefore, the variation in inner temperature (body core) of the animals acts as a signal of

physiological changes, more specifically as an attempt to increase the heat dissipation to the extremities of the body (NASCIMENTO, 2010).

The observation of the varied scenarios for the thermal variables t_{db} and RR can lead us to infer that, under high temperature and humidity conditions, birds face difficulties in transferring excess heat to the environment, leading to increases in body temperature and thermal discomfort, as well as decreases in production (SOUZA, 2005). Oliveira et al. (2006) stated that the birds' capacity to cope with heat is inversely proportional to the relative humidity of the air. The higher the RH, the higher the difficulty in removing heat from the airways to the environment, which leads to a higher respiratory rate.

Equation n° 24, table 3 represents the empirical model of best fit, this model presented adequate statistical indexes that allow its application in practical situations since it was evidenced a relationship between the bird's physiology and the variable that regulate this response (t_{db}). Under conditions of high temperature stress, birds increase their respiratory rate by up to ten times their regular rate in response to thermal discomfort, indicating the use of cooling evaporation mechanisms (SANTOS et al., 2006). In scenarios of high temperatures associated with high relative air humidity, birds can develop respiratory alkalosis (BORGES et al., 2003).

$$RR = -101.8 (16.8) + 5.67(0.60) \cdot t_{db} \quad (5)$$

Table 2. Performance (accuracy and precision) of multivariate and simple models adjusted to distinct analysis methodologies (empirical model, fuzzy and artificial neural networks) and their respective statistical indices for the prediction of cloacal temperature (t_{cloacal})

Model Number	Model ¹	Conventional					Fuzzy logic					ANN					
		F-Test	MAE	SD	RMSE	R ²	MAPE	MAE	SD	RMSE	R ²	MAPE	MAE	SD	RMSE	R ²	MAPE
1	$t_{\text{cloacal}} = f(t_{\text{db}}, \text{RH}, \text{V})$	N.S.	-	-	-	-	-	0.15	0.11	0.04	0.98	0.38	0.13	0.09	0.05	0.80	0.32
2	$t_{\text{cloacal}} = f(t_{\text{db}}, t_{\text{sur}})$	N.S.	-	-	-	-	-	0.21	0.15	0.26	0.70	0.51	0.31	0.22	0.39	0.30	0.76
3	$t_{\text{cloacal}} = f(t_{\text{db}}, \text{RH}, t_{\text{sur}})$	N.S.	-	-	-	-	-	0.23	0.16	0.31	0.62	0.57	0.36	0.25	0.44	0.29	0.89
4	$t_{\text{cloacal}} = f(t_{\text{db}}, \text{RH}, \text{V}, t_{\text{sup}})$	N.S.	-	-	-	-	-	0.12	0.09	0.19	0.80	0.31	0.29	0.20	0.40	0.18	0.72
5	$t_{\text{cloacal}} = f(\text{THI}, t_{\text{sur}})$	N.S.	-	-	-	-	-	0.19	0.13	0.24	0.74	0.46	0.80	0.57	0.93	0.00	2.00
6	$t_{\text{cloacal}} = f(\text{BGHI}, t_{\text{sur}})$	N.S.	-	-	-	-	-	0.21	0.15	0.30	0.55	0.53	0.25	0.18	0.31	0.61	0.62
7	$t_{\text{cloacal}} = f(\text{RHL}, t_{\text{sur}})$	N.S.	-	-	-	-	-	0.21	0.15	0.27	0.68	0.53	0.43	0.31	0.57	0.55	1.07
8	$t_{\text{cloacal}} = f(\text{H}, t_{\text{sur}})$	N.S.	-	-	-	-	-	0.14	0.10	0.22	0.74	0.35	0.31	0.22	0.39	0.30	0.76
9	$t_{\text{cloacal}} = f(t_{\text{bs}})$	***	0.35	0.34	0.44	0.36	0.88	-	-	-	-	-	-	-	-	-	-
10	$t_{\text{cloacal}} = f(\text{THI})$	***	0.38	0.29	0.47	0.28	0.93	-	-	-	-	-	-	-	-	-	-
11	$t_{\text{cloacal}} = f(\text{BGHI})$	***	0.38	0.29	0.47	0.27	0.94	-	-	-	-	-	-	-	-	-	-
12	$t_{\text{cloacal}} = f(\text{RHL})$	***	0.37	0.29	0.47	0.27	0.93	-	-	-	-	-	-	-	-	-	-
13	$t_{\text{cloacal}} = f(\text{H})$	***	0.38	0.28	0.48	0.25	0.95	-	-	-	-	-	-	-	-	-	-
14	$t_{\text{cloacal}} = f(t_{\text{sur}})$	***	0.40	0.25	0.50	0.19	0.99	-	-	-	-	-	-	-	-	-	-
15	$t_{\text{cloacal}} = f(\text{RR})$	***	0.35	0.33	0.44	0.36	0.86	-	-	-	-	-	-	-	-	-	-

Legend: MAE (mean absolute error); SD (standard deviation); RMSE (root mean square error); MAPE (mean absolute percentage error); R² (coefficient of determination). F-test: Fisher, Anova, $p > 0.05$ (N.S.); *** ($p < 0.05$).

¹Minimum and maximum values for the physiological variable t_{cloacal} (°C) according to the range of air temperature evaluated: t_{cloacal} [38.8; 42.1]; t_{db} [20;36].

t_{db} : dry bulb temperature (°C), RH: relative humidity (%), V: Air velocity (m s^{-1}), THI: temperature humidity index (dimensionless), BGHI: Black globe-humidity index (dimensionless), RHL: radiant heat load (W m^{-2}), H: enthalpy ($\text{kJ kg}_{\text{dry air}}^{-1}$), t_{cloacal} : cloacal temperature (°C), t_{sur} : temperature of bird's surface (°C), RR: respiratory rate (mov min^{-1}).

Table 3. Performance (accuracy and precision) of multivariate and simple models adjusted to distinct analysis methodologies (empirical model, fuzzy and artificial neural networks) and their respective statistical indices for the prediction of respiratory rate (RR)

Model Number	Model	Conventional					Fuzzy logic					ANN					
		F-Test	MAE	SD	RMSE	R ²	MAPE	MAE	SD	RMSE	R ²	MAPE	MAE	SD	RMSE	R ²	MAPE
16	RR = f(t _{db} , RH, V)	N.S.	-	-	-	-	-	1.80	1.27	3.52	0.99	2.89	1.98	1.40	2.59	0.99	4.98
17	RR = f(t _{db} , t _{sur})	N.S.	-	-	-	-	-	5.85	4.14	7.41	0.96	12.94	5.51	3.90	7.76	0.96	9.99
18	RR = f(t _{db} , RH, t _{sur})	N.S.	-	-	-	-	-	6.27	4.43	10.66	0.94	9.68	11.12	7.86	12.50	0.89	28.19
19	RR = f(t _{db} , RH, V, t _{sup})	N.S.	-	-	-	-	-	2.99	2.11	4.04	0.99	5.38	4.03	2.85	5.37	0.98	8.76
20	RR = f(THI, t _{sur})	N.S.	-	-	-	-	-	4.09	2.89	5.57	0.98	7.99	4.10	2.90	5.80	0.98	8.65
21	RR = f(BGHI, t _{sur})	N.S.	-	-	-	-	-	4.56	3.23	5.95	0.98	8.40	6.85	4.84	9.19	0.97	12.75
22	RR = f(RHL, t _{sur})	N.S.	-	-	-	-	-	5.61	3.97	7.71	0.96	10.84	6.54	4.63	11.20	0.95	10.71
23	RR = f(H, t _{sur})	N.S.	-	-	-	-	-	3.72	2.63	4.83	0.98	7.35	3.75	2.65	4.76	0.98	8.34
24	RR = f(t _{bs})	***	16.84	38.81	22.79	0.74	34.59	-	-	-	-	-	-	-	-	-	-
25	RR = f(THI)	***	25.14	32.66	30.93	0.52	53.82	-	-	-	-	-	-	-	-	-	-
26	RR = f(BGHI)	***	25.05	32.80	30.78	0.53	53.83	-	-	-	-	-	-	-	-	-	-
27	RR = f(RHL)	***	24.94	32.99	30.57	0.53	53.17	-	-	-	-	-	-	-	-	-	-
28	RR = f(H)	***	22.58	33.39	30.14	0.55	47.54	-	-	-	-	-	-	-	-	-	-
29	RR = f(t _{cloacal})	***	26.59	26.40	35.80	0.36	52.31	-	-	-	-	-	-	-	-	-	-
30	RR = f(t _{sur})	***	28.04	28.38	34.87	0.39	57.59	-	-	-	-	-	-	-	-	-	-

Legend: MAE (mean absolute error); SD (standard deviation); RMSE (root mean square error); MAPE (mean absolute percentage error); R² (coefficient of determination). F-test: Fisher, Anova, p>0.05 (N.S.); *** (p<0.05).

¹Minimum and maximum values for the physiological variable RR (mov min⁻¹) according to the range of air temperature evaluated: RR [21; 191]; t_{db} [20;36].

t_{db}: air dry-bulb temperature (°C), RH: relative humidity (%), V: air velocity (m s⁻¹), THI: temperature humidity index (dimensionless), BGHI: black globe-humidity index (dimensionless), RHL: radiant heat load (W m⁻²), H: enthalpy (kJ kg_{dry air}⁻¹), t_{cloacal}: cloacal temperature (°C), t_{sur}: temperature of bird's surface (°C), RR: respiratory rate (mov min⁻¹).

The fuzzy inference is composed of a rule system (Table 4) based on the mean values of the data obtained experimentally, as well as on experts' knowledge. Logical connectives form each rule (IF, AND, OR, THEN) and both parts by antecedents and consequents. For instance: If x is A and y is B then z is C , in where A , B and C are fuzzy sets, x and Y are input variables, z is the output variable, "If x is A and y is B " is the antecedent and "THEN z is C " is the consequent (TANAKA, 1997; PEDRYCZ, GOMIDE, 1998). According to the combinations of the input data, 30 rules were defined and a weighting factor of 1 was attributed for each one of them.

In the fuzzy logic structure, the relationship between a universal set U and a fuzzy subset A is characterized by a pertinence curve or function which assigns a degree of pertinence from 0 to 1 to each element in A . Thus, infinite values can be attributed to the subset, not only "belongs to" or "does not belong to" (SCHIASSI et al., 2008).

The intervals declared for the input variables were: t_{db} [20; 36]; RH [40; 60]; V [0.2; 1.4] and for the output variables $t_{cloacal}$ [39.0; 41.5] and RR [24; 163.5]. All the variables were represented by triangular pertinence curves (Figures 2 and 3), for they best represent both the classes of input and output data, a solution used by many authors (PANDORFI et al., 2011; SCHIASSI et al., 2008; TOLON et al., 2010). The intervals adopted were based on the t_{db} , RH and V ranges established during the experiment and through consultation of experts and literature in the area (CASTILHO et al., 2015; MARUCCI et al., 2013; EBEID et al., 2012; OLIVEIRA et al., 2011; MUTAF et al., 2008; JÁCOME et al., 2007; OLIVEIRA et al., 2006; VITORASSO, PEREIRA, 2009; TINÔCO, 2004; YANAGI JÚNIOR et al., 2002).

Table 4. Composition of the rule system used in the fuzzy inference for the input variables: air dry-bulb temperature (t_{db}), air relative humidity (RH), air velocity (V) and output variables: cloacal temperature ($t_{cloacal}$) and respiratory rate (RR)

RULES¹	
1	If (t_{db} is very low) and (RH is low) and (V is low) Then ($t_{cloacal}$ is MF 3) (RR is MF 1)
2	If (t_{db} is very low) and (RH is low) and (V is medium) Then ($t_{cloacal}$ is MF 3) (RR is MF 3)
3	If (t_{db} is very low) and (RH is low) and (V is high) Then ($t_{cloacal}$ is MF 4) (RR is MF 1)
4	If (t_{db} is very low) and (RH is high) and (V is low) Then ($t_{cloacal}$ is MF 2) (RR is MF 3)
5	If (t_{db} is very low) and (RH is high) and (V is medium) Then ($t_{cloacal}$ is MF 4) (RR is MF 3)
6	If (t_{db} is very low) and (RH is high) and (V is high) Then ($t_{cloacal}$ is MF 1) (RR is MF 1)
7	If (t_{db} is low) and (RH is low) and (V is low) Then ($t_{cloacal}$ is MF 8) (RR is MF 4)
8	If (t_{db} is low) and (RH is low) and (V is medium) Then ($t_{cloacal}$ is MF 3) (RR is MF 5)
9	If (t_{db} is low) and (RH is low) and (V is high) Then ($t_{cloacal}$ is MF 6) (RR is MF 4)
10	If (t_{db} is low) and (RH is high) and (V is low) Then ($t_{cloacal}$ is MF 3) (RR is MF 4)
11	If (t_{db} is low) and (RH is high) and (V is medium) Then ($t_{cloacal}$ is MF 4) (RR is MF 3)
12	If (t_{db} is low) and (RH is high) and (V is high) Then ($t_{cloacal}$ is MF 7) (RR is MF 7)
13	If (t_{db} is medium) and (RH is low) and (V is low) Then ($t_{cloacal}$ is MF 3) (RR is MF 3)
14	If (t_{db} is medium) and (RH is low) and (V is medium) Then ($t_{cloacal}$ is MF 1) (RR is MF 2)
15	If (t_{db} is medium) and (RH is low) and (V is high) Then ($t_{cloacal}$ is MF 3) (RR is MF 4)
16	If (t_{db} is medium) and (RH is high) and (V is low) Then ($t_{cloacal}$ is MF 2) (RR is MF 5)
17	If (t_{db} is medium) and (RH is high) and (V is medium) Then ($t_{cloacal}$ is MF 5) (RR is MF 2)
18	If (t_{db} is medium) and (RH is high) and (V is high) Then ($t_{cloacal}$ is MF 5) (RR is MF 7)
19	If (t_{db} is high) and (RH is low) and (V is low) Then ($t_{cloacal}$ is MF 8) (RR is MF 6)
20	If (t_{db} is high) and (RH is low) and (V is medium) Then ($t_{cloacal}$ is MF 10) (RR is MF 6)
21	If (t_{db} is high) and (RH is low) and (V is high) Then ($t_{cloacal}$ is MF 5) (RR is MF 8)
22	If (t_{db} is high) and (RH is high) and (V is low) Then ($t_{cloacal}$ is MF 7) (RR is MF 8)
23	If (t_{db} is high) and (RH is high) and (V is medium) Then ($t_{cloacal}$ is MF 6) (RR is MF 9)
24	If (t_{db} is high) and (RH is high) and (V is high) Then ($t_{cloacal}$ is MF 2) (RR is MF 7)
25	If (t_{db} is very high) and (RH is low) and (V is low) Then ($t_{cloacal}$ is MF 10) (RR is MF 10)
26	If (t_{db} is very high) and (RH is low) and (V is medium) Then ($t_{cloacal}$ is MF 11) (RR is MF 11)
27	If (t_{db} is very high) and (RH is low) and (V is high) Then ($t_{cloacal}$ is MF 9) (RR is MF 12)
28	If (t_{db} is very high) and (RH is high) and (V is low) Then ($t_{cloacal}$ is MF 9) (RR is MF 14)
29	If (t_{db} is very high) and (RH is high) and (V is medium) Then ($t_{cloacal}$ is MF 9) (RR is MF 13)
30	If (t_{db} is very high) and (RH is high) and (V is high) Then ($t_{cloacal}$ is MF 12) (RR is MF 13)

¹MF: membership function

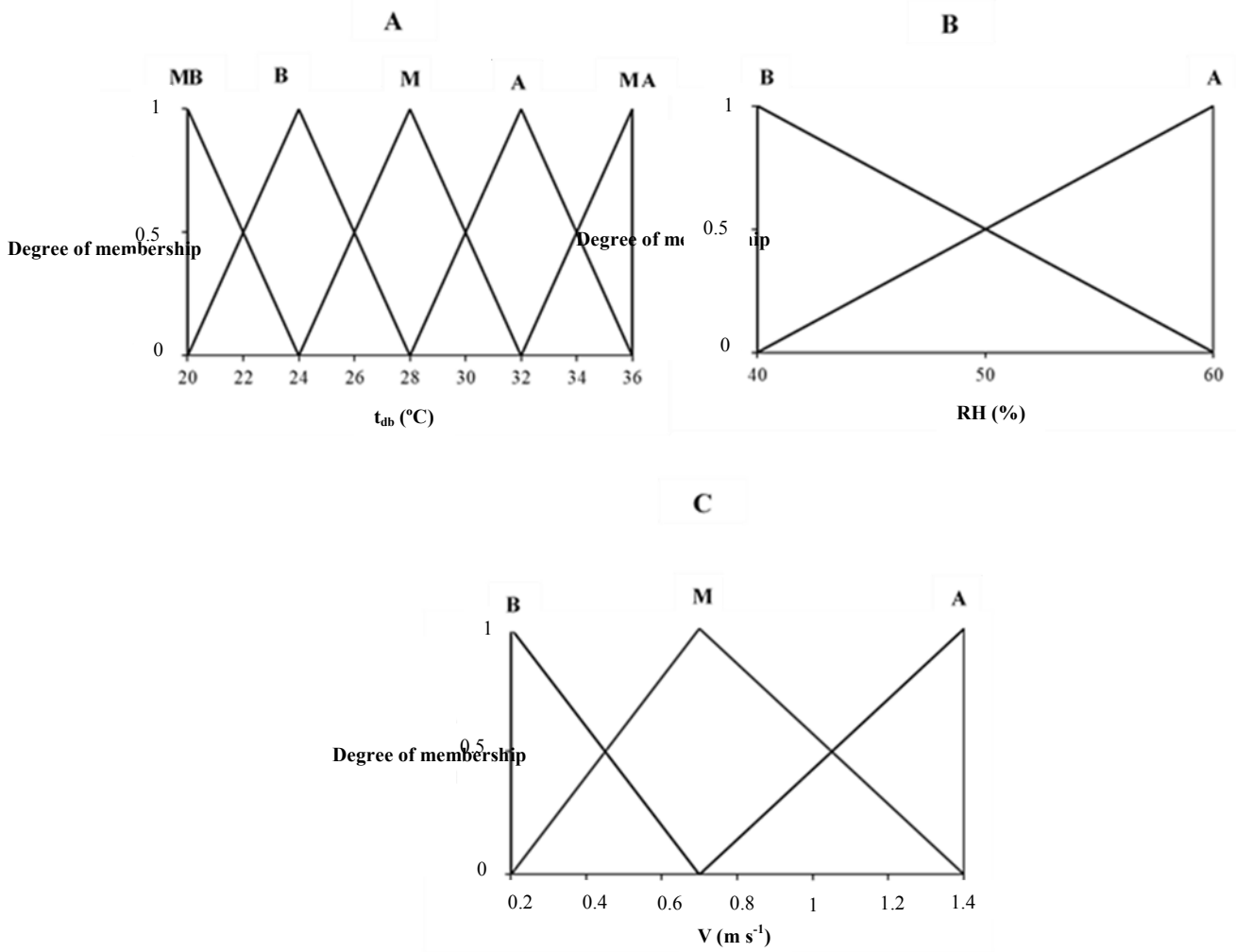


Figure 3. Membership function for the input variables: (A) air dry-bulb temperature (t_{db} , °C), (B) air relative humidity (RH, %), and (C) air velocity (V , $m\ s^{-1}$). VL: very low; L: low; M: medium; H: high; VH: very high.

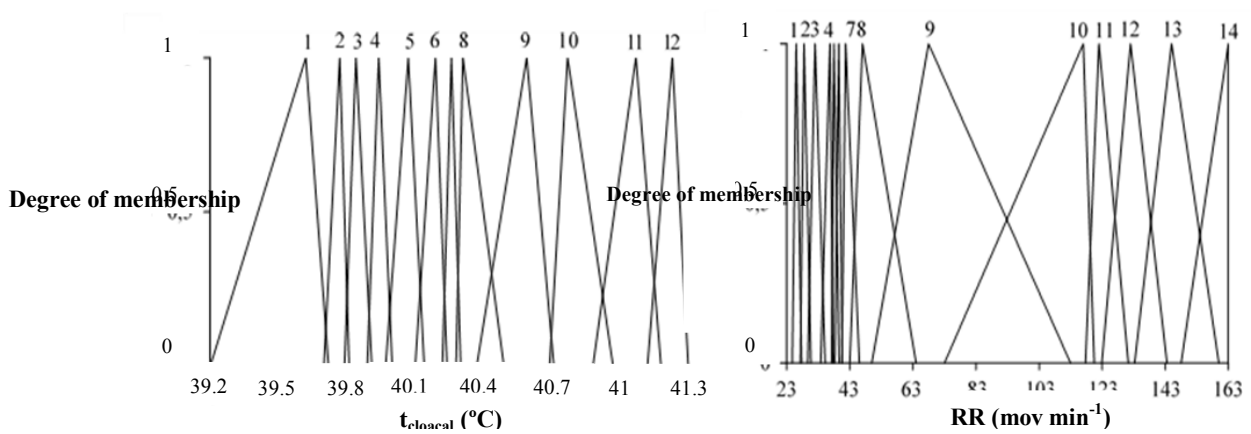


Figure 4. Membership function for the output variables: (A) cloacal temperature ($t_{cloacal}$, °C) and (B) respiratory rate (RR, $mov\ min^{-1}$).

Figures 5 and 6 show the adjustments using artificial intelligence methodologies applied to t_{cloacal} ($^{\circ}\text{C}$) and RR (mov min^{-1}), respectively. Figure 5 shows the functional relationship between the predicted and observed t_{cloacal} values by the ANN of the best performance when the whole dataset was used. The ANN methodology applied to t_{cloacal} in this study generated a model with a significant correlation (0.8002). This result corroborates the studies of Ferraz et al. (2014), which used ANN to predict the body mass of chicks under different heat stress conditions and obtained a correlation coefficient R^2 of 0.9993, confirming the high accuracy of artificial intelligence to predict poultry responses. Bahuti et al. (2018) trained an ANN to predict productive and physiological responses in poultry subject to thermal stress and observed an R^2 of 0.87 for t_{cloacal} , which is similar to the value of 0.8 obtained in the present study.

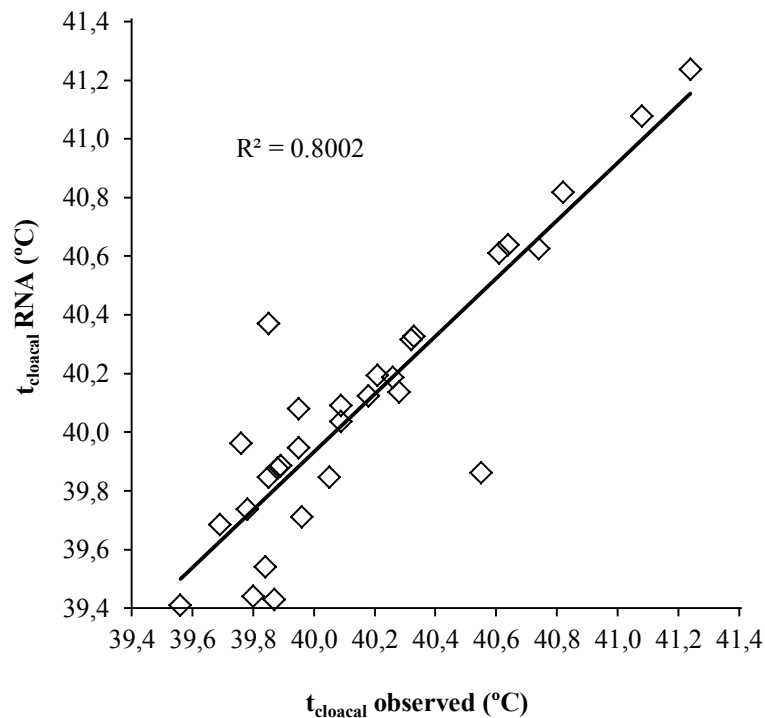


Figure 5. The functional relationship between observed and ANN-predicted cloacal temperature (t_{cloacal}) ($^{\circ}\text{C}$).

In Figure 6, we can observe an R^2 of 0.9933 for RR, indicating adequate precision of the fuzzy system used in the prediction of such variables.

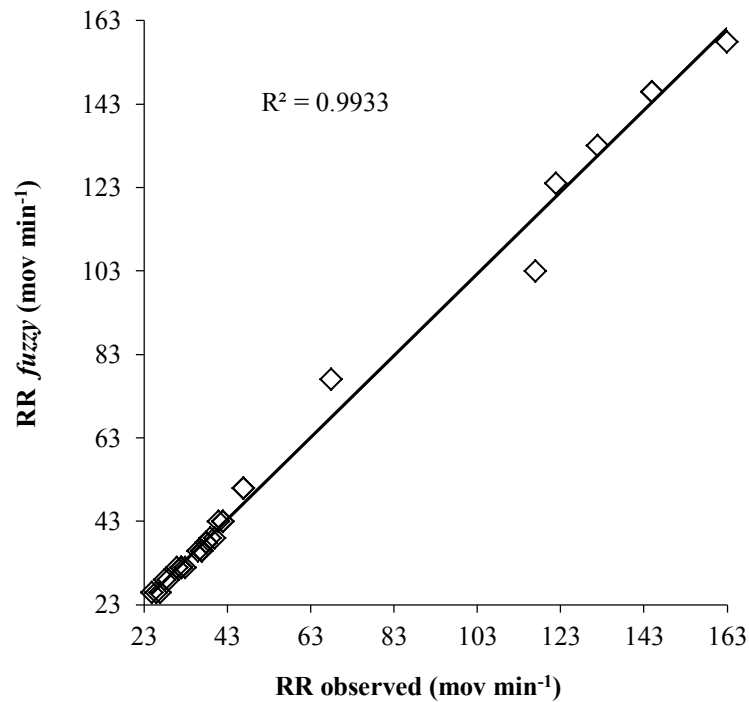


Figure 6. The functional relationship between observed and fuzzy-predicted respiratory rate (RR).

Although Aerts et al. (2003) and Brown-Brandl et al. (2005) described systems to support decision-making in animal production systems, few of them are destined to welfare estimates. However, experiments carried out in climatic chambers show results with R^2 higher than 86%. Pereira et al. (2008) developed a system to support decision-making based on the fuzzy set theory and estimate well-being in heavy type laying hens using the birds' response to the environment.

HERNÁNDEZ-JULIO et al., 2020 when studying reproductive aspects of copepod crustaceans, applied the same methodology based on fuzzy sets using clusters and pivot tables and other methodologies such as ANNs and Adaptive Neuro-Fuzzy Inference System. In this way, they found the same significant configuration of the fuzzy methodology compared to the other applied methodologies, as well as in this work, where it was found a better adjustment of the fuzzy systems compared to ANNs for the physiological variable RR.

They concluded that the implementation of the fuzzy methodology for the prediction of welfare in heavy type laying hens can be of help when decision regarding activation of cooling systems in poultry buildings needs to be made. It showed more realistic results as a function of environmental and behavioral situations observed in those buildings.

4. CONCLUSION

The development of artificial intelligence-based models, fuzzy and ANN, especially models based on the climatic variables t_{db} , RH and V, resulted in adequate correlation coefficients for the prediction of the physiological variables $t_{cloacal}$ and RR. For the $t_{cloacal}$ the methodology with the best adjustment was the ANNs, and for the RR, the fuzzy logic stood out.

Both $t_{cloacal}$ and RR physiological variables are directly influenced by the t_{db} , RH and V meteorological variables, thus not being influenced by bioclimatic indices and thermodynamic properties.

In the case of commercial production, the application of these methodologies can assist in decision-making processes related to the activation of refrigeration systems in poultry buildings to maintain thermoneutral conditions.

Acknowledgments

To Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), to Fundação de Amparo à Pesquisa do Estado de Minas Gerais (FAPEMIG), and to Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES), for financial support.

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**ARTICLE 3 - EMPIRICAL AND ARTIFICIAL INTELLIGENCE-BASED MODELS
APPLIED TO THE PREDICTION OF SURFACE TEMPERATURE OF LAYING
HENS**

(DRAFT VERSION)

Article prepared for journal submission *Computers and Electronics in Agriculture*.

EMPIRICAL AND ARTIFICIAL INTELLIGENCE-BASED MODELS APPLIED TO THE PREDICTION OF SURFACE TEMPERATURE OF LAYING HENS

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ABSTRACT: The present study aimed to evaluate the use of empirical and artificial intelligence-based models to predict the surface temperature of laying hens subject to different thermal environments. Empirical and artificial intelligence-based models were developed: fuzzy logic and artificial neural networks (ANNs). The best models were composed of the input variables air dry-bulb temperature (t_{db}), air relative humidity (RH) and air velocity (V), with surface temperature as the output variable ($t_{surface}$). The use of artificial intelligence-based models resulted in a multivariate model with a better fit, with a coefficient of determination of 0.9983 for fuzzy logic and 0.9728 for trained ANNs. In the face of the different thermal scenarios evaluated, which represent the reality observed inside contemporary poultry systems, we can infer that new methodologies of fuzzy modeling and ANN resulted in models with a better fit. It indicates the importance of using novel methodologies for microclimate control inside poultry buildings.

Keywords: animal bioclimatology; physiology; computer modeling; poultry farming.

1. INTRODUCTION

Thermal-related variables directly influence animal welfare and comfort in intensive egg production systems. They also affect the thermal balance inside poultry buildings and the expression of animals' natural behavior, which impacts the performance of laying hens (MASHALY et al., 2004; CANDIDO et al., 2015). Animal productivity is highest in thermoneutral environments, in which birds maintain their body temperature within the thermoneutral zone (TNZ), i.e. when food energy is not used to compensate for thermal deviations (CIGR, 2002). The ideal ambient temperature for the thermal comfort of birds is between 21 and 28°C (CASTILHO et al., 2015). Ferreira (2016) claims that adult laying hens are more productive when raised in environments whose relative humidity ranges between 40% and 70%.

Sensible heat loss in birds is highly efficient in areas without feathers due to the thermal insulation provided by the thick feather cover on most of their bodies. In featherless areas, blood flow increases when birds are exposed to thermal stress. Laying hens do not possess sweat glands, and their sweating capacity is non-existent. Thus, they lose excess heat mainly by evaporating water through respiration and releasing heat from surfaces such as combs, dewlaps, shanks and featherless areas under their wings.

Animals behave as thermodynamic systems that continuously exchange heat with their surroundings (FERREIRA, 2016). In birds, the sensation of heat is produced by specialized cells that work as peripheral thermoreceptors, taking those sensations to the central nervous system. The anterior hypothalamus is responsible for the sensation of heat in hot environments (ABREU, ABREU, 2004). Therefore, hyperthermia-induced stress is traditionally characterized by an increase in body core temperature. On the other hand, surface body temperature decreases as a result of peripheral vasoconstriction, which is part of the broader stress-induced hyperthermia process (BUSNARDO et al., 2010). The physiological temperature controlled by the thermo-regulating center is, therefore, an indicator of welfare for chronic stress. Chronic stress increases body core temperature, while processes that dissipate or conserve heat of the body core interferes directly with surface temperature (HERBORN et al., 2018).

The surface body temperature of laying hens can be used to evaluate the microclimate inside poultry buildings, as well as their influence on other physiological variables. Such evaluation consists of the quantification of changes in blood flow and minimal variations in body temperature to infer thermal comfort – or discomfort – in birds. Surface temperature is

an easily measurable and noninvasive parameter since it is taken with the use of infrared thermography.

In egg production environments, the use of mathematical and computer modeling techniques is a strategy that contributes to the prediction of physiological responses in different thermal scenarios. The artificial neural networks (ANNs) and fuzzy logic are among the most used and tested artificial intelligence methodologies to perform tasks or solve problems using a knowledge basis (SCHIASSI et al., 2014). In this context, the present study aimed to evaluate the use of empirical and artificial intelligence-based models to predict the surface temperature of laying hens subject to different thermal environments.

2. MATERIAL AND METHODS

All experimental procedures involving animals were previously approved by the Ethics Committee on Animal Use (CEUA) of the Federal University of Lavras, under protocol N° 079/17.

Data were collected using four thermal environment-controlled wind tunnels equipped with heating and air moistening function. A total of ninety Hy-line laying hens, 28 weeks old, in peak production were used. The animals were subjected to a factorial combination of five air dry-bulb temperatures (t_{db} : 20, 24, 28, 32 and 36°C), two air relative humidities (RH: 40 and 60%) and three air velocities (V : 0.2, 0.7 and 1.4 ms^{-1}), totaling 30 treatments. Each laying hen was exposed to thermal challenge only once. Birds were allowed seven days for adaptation to facilities before the thermal challenge. During the acclimatization period, the birds were subject to a room temperature of $23.2 \pm 0.1^\circ C$ and RH of $60.5 \pm 0.8\%$, conditions of thermal comfort for them (CURTIS, 1983; ALBRIGHT, 1990; BAËTA, SOUZA, 2010), i.e., within the interval between 18 to 25°C for t_{db} and 40 to 60% for RH (MARUCCI et al., 2013; JÁCOME et al., 2007).

The surface temperature ($t_{surface}$) was evaluated through a thermographic camera (model TI 55, Fluke, accuracy $0.05^\circ C$). Laying hens were thermally challenged for at least 180 minutes or until stabilization of cloacal temperature but not exceeding the maximum of 360 minutes in order to respect animal welfare. It corroborates with Yanagi Júnior et al. (2002) study with laying hens exposed to acute stress ($35^\circ C$), in which the time required for stabilization of cloacal temperature ranged from 106 to 185 minutes. We also measured the physiological variables cloacal temperature ($t_{cloacal}$) and respiratory rate RR). The $t_{cloacal}$ was measured using a digital thermometer (accuracy $\pm 0.2^\circ C$), and RR was measured by counting respiratory movements for 15 seconds and then multiplying it by four.

The birds were allotted to a 5 x 2 x 3 factorial completely randomized design (CRD) with three replicates. The analysis of variance and adjustment of conventional models were performed using R Core Team software (2016). Fuzzy logic and ANN models were developed using the software MATLAB® version R2011b (7.13.0.564).

The following statistical indices were used to measure the goodness-of-fit of each model: mean absolute error (MAE), root mean square error (RMSE), standard deviation (SD) and mean absolute percentage error (MAPE).

2.1. Artificial intelligence-based methodology 1: Fuzzy logic

The Mamdani inference method was used to develop the fuzzy systems, which assume that each rule is a fuzzy conditional proposition, and different fuzzy relationships can be derived from it (AMENDOLA, SOUZA, 2004). It has as an outcome a fuzzy set originated from the combination of input values and their respective pertinence degrees using the minimum operator, followed by the superposition of the rules with the use of the maximum operator. Defuzzification was carried out by applying the Center of Gravity method (Centroid or Center of Area), which considers all output possibilities, turning the fuzzy set originated by the inference into a numeric value. Thus, it corresponds to the functional link between fuzzy regions and the expected value (AMENDOLA, SOUZA, 2004).

Fuzzy inference systems were developed based on the methodology of Hernández-Julio et al. (2019, 2018 and 2019). A total of 13 steps were taken (HERNÁNDEZ-JULIO et al., 2019) as follows: 1) dataset identification: dataset consists of 90 instances and four attributes or variables (three input variables and one output variables), with no missing values, and attributes were integer and real; 2) data preparation (input); 3) review of existing models: searching for related studies and comparing them with our results and other results in the literature; 4) determination of the optimal number of clusters; 5) defining the minimum and the maximum number of clusters; 6) random permutation of inputs and outputs; 7) fuzzy process: the clustering method was used. Ward's method was used to classify each input and output variable. We selected the Euclidian distance as a distance measurement. As stated by Hernández-Julio et al. (2019), the fuzzy knowledge base is obtained in this step; 8) random sampling; 9) creating dynamic tables to accelerate search speed and filter information; 9.1) combining clusters datasets by using the commands “nchoosek” and “unique” for matrices; 9.2) establishing the fuzzy rules with the command “unique”; 10) elaborating the Decision Support System based on the fuzzy set theory by using the software Matlab® (The Mathworks

Inc., R2011b). The following components were used: definition of linguistic variables, the set of rules, the number of sets and the values of the Membership function for each variable. We used the proposed algorithms mentioned in the literature (HERNÁNDEZ-JULIO et al., 2019); 11) evaluating the performance of fuzzy inference systems through the following statistical indices: mean absolute error (MAE), root mean square error (RMSE), standard deviation (SD) and mean absolute percentage error (MAPE).

Dynamic tables were applied for each input variable (three variables; item 4). Most of the input variables were larger than 20 fuzzy sets; hence, the recommendation made by the author of the framework was followed (HERNÁNDEZ-JULIO et al., 2019). The rounded square root of every input variable higher than 20 was determined. The optimal number of clusters for each input and output variable can be seen in Table 1.

Table 1. The optimal number of clusters for each input and output variable

	Input variables			Output variables
	t_{db} (°C)	RH (%)	V ($m s^{-1}$)	$t_{surface}$ (°C)
Number of lines	72	68	3	86
Square root (rounded)	8	8	3	9

t_{db} : air dry-bulb temperature (°C), RH: air relative humidity (%), V: air velocity ($m s^{-1}$) and $t_{surface}$: temperature of bird's surface (°C).

2.2. Artificial intelligence-based methodology 2: Artificial Neural Networks (ANNs)

The use of ANNs has advantages inherent to their particular structure and properties: they are flexible, adaptable, and can be applied to a wide range of problems and situations – for prediction of physiological variables such as body temperature, for instance. The methodology to build the ANN structure used in this study was established by Hernández-Julio et al. (2014) and Ponciano Ferraz et al. (2014). Feedforward was the topology of the ANNs used. Layers represent it; in the input layer, the neurons receive the input signal, and in the output layer, the results of the processing are sent to the ANN. The Levenberg-Marquardt backpropagation algorithm was used; it consists of a supervised learning algorithm used for pattern recognition and comparison with the ANN output. The network architecture relates hidden layers, the direction of the synaptic connections between neurons, the number of neurons in the layers and their respective activation functions, learning rate, synaptic network weights and neuron deviations.

Two subsets were randomly separated: training (70% of the data) and validation (30% of the data). The first subset was used to obtain the optimal weights associated with the neurons. The validation set was used to reach the ideal number of hidden neurons or to determine an endpoint to the backpropagation algorithm. The number of hidden neurons varied according to each methodology. The configurations with the highest accuracy, i.e., coefficients of determination (R^2) and the smallest mean square error (MSE) were selected.

2.3. Thermal comfort indexes and thermodynamic property (enthalpy)

Simple linear regression models and a multivariate model were adjusted for the physiological variable surface temperature ($^{\circ}\text{C}$); thermal comfort indexes: black globe-humidity index – BGHI (BUFFINGTON et al., 1981), temperature-humidity index – THI (THOM, 1959), radiant heat load – RHL (ESMAY, 1982); enthalpy - H (ALBRIGHT, 1990) and environment variables: t_{db} ($^{\circ}\text{C}$), RH (%) and V (m s^{-1}).

Equations 1, 2, and 3 were used for determination of thermal comfort indexes, while equation 5 was used to calculate enthalpy:

$$\text{BGHI} = t_{bg} + 0.36 \cdot t_{dp} + 41.5 \quad (1)$$

where:

t_{bg} = black-globe temperature ($^{\circ}\text{C}$);

t_{dp} = air dew-point temperature ($^{\circ}\text{C}$).

$$\text{THI} = t_{db} + 0.36 \cdot t_{dp} + 41.5 \quad (2)$$

where:

t_{db} : air dry-bulb temperature ($^{\circ}\text{C}$);

t_{dp} : air dew-point temperature ($^{\circ}\text{C}$).

$$\text{RHL} = \sigma \cdot (\text{MRT})^4 \quad (3)$$

where:

σ : Stefan-Boltzmann constant ($5.67 \times 10^{-8} \text{ W m}^{-2} \text{ K}^{-4}$);

MRT: mean radiant temperature (K) (equation number 4).

$$\text{MRT} = 100 \cdot \sqrt[4]{2.51 \cdot \sqrt{V} \cdot (T_{\text{bg}} - T_{\text{db}}) + \left(\frac{T_{\text{bg}}}{100}\right)} \quad (4)$$

where:

MRT: mean radiant temperature (K);

V: air velocity (m s^{-1});

T_{bg} : black-globe temperature (K);

T_{db} : ambient temperature (K).

$$H = 1.006 \cdot t_{\text{db}} + W \cdot (2501 + 1.805 \cdot t_{\text{db}}) \quad (5)$$

where:

t_{db} : air dry-bulb temperature ($^{\circ}\text{C}$);

W: humidity ratio ($\text{kg}_{\text{water vapor}} \text{kg}_{\text{dry air}}^{-1}$)

$$W = \frac{(0.622 \cdot e_a)}{(P_{\text{atm}} - e_a)} \quad (6)$$

where:

e_a : actual vapor pressure (kPa)

P_{atm} : atmospheric pressure (kPa)

3. RESULTS AND DISCUSSION

The statistical indices used to check precision for each empirical model are shown in table 1. The conventional model with the best precision was model number 2 -adjusted as a function of t_{db} (equation 6) - with values of MAE, SD, RMSE, R^2 , and MAPE of 0.55, 4.00, 0.57, 0.98 and 1.79, respectively. The statistical indices of equations adjusted as a function of other environmental variables (t_{db} , RH and V), thermal comfort indexes (BGHI, THI, RHL) and enthalpy (H) had potential to be used. Empirical models adjusted as a function of t_{cloacal} and RR had the least precise statistical indices, limiting their use for predicting t_{surface} .

Table 2. Models adjusted to distinct data analysis methodologies (empirical, fuzzy and artificial neural networks) and their respective statistical indices for the prediction of surface temperature (t_{surface}) of laying hens

N° Mod.	Model ¹	Conventional						Fuzzy logic				ANN					
		F-test	MAE	SD	RMSE	R ²	MAPE	MAE	SD	RMSE	R ²	MAPE	MAE	SD	RMSE	R ²	MAPE
1	$t_{\text{surface}} = f(t_{\text{db}}, \text{RH}, \text{V})$	***	1.33	4.01	1.54	0.87	4.29	0.14	0.10	0.18	1.00	0.46	0.43	0.30	0.56	0.97	1.43
2	$t_{\text{surface}} = f(t_{\text{db}})$	***	0.55	4.00	0.57	0.98	1.79	-	-	-	-	-	-	-	-	-	-
3	$t_{\text{surface}} = f(\text{THI})$	***	1.34	3.99	1.58	0.86	4.37	-	-	-	-	-	-	-	-	-	-
4	$t_{\text{surface}} = f(\text{BGHI})$	***	1.35	3.98	1.60	0.86	4.38	-	-	-	-	-	-	-	-	-	-
5	$t_{\text{surface}} = f(\text{RHL})$	***	1.43	3.95	1.69	0.84	4.62	-	-	-	-	-	-	-	-	-	-
6	$t_{\text{surface}} = f(\text{H})$	***	1.85	3.71	2.15	0.75	6.05	-	-	-	-	-	-	-	-	-	-
7	$t_{\text{surface}} = f(t_{\text{cloacal}})$	***	3.26	1.89	3.84	0.19	10.84	-	-	-	-	-	-	-	-	-	-
8	$t_{\text{surface}} = f(\text{RR})$	***	2.72	2.71	3.32	0.39	9.10	-	-	-	-	-	-	-	-	-	-

MAE (mean absolute error); SD (standard deviation); RMSE (root mean square error); MAPE (mean absolute percentage error); R² (coefficient of determination). F-test: Fisher, Anova, $p > 0.05$ (N.S.); *** ($p < 0.05$).

¹Minimum and maximum values for the physiological variable t_{surface} (°C) according to the range of air temperature evaluated: t_{surface} [23.2; 38.9]; t_{db} [20;36].

t_{db} : air dry-bulb temperature (°C), RH: air relative humidity (%), V: air velocity ($\text{m}\cdot\text{s}^{-1}$), THI: temperature humidity index (dimensionless), BGHI: black globe-humidity index (dimensionless), RHL: radiant heat load ($\text{W}\cdot\text{m}^{-2}$), H: enthalpy ($\text{kJ}\cdot\text{kg}_{\text{dry air}}^{-1}$), t_{cloacal} : cloacal temperature (°C), t_{surface} : temperature of bird's surface (°C), RR: respiratory rate ($\text{mov}\cdot\text{min}^{-1}$).

Models 1 to 8 represent, respectively, the best empirical models adjusted for the prediction of t_{surface} as a function of t_{db} , RH and V; THI; BGHI; RHL; H; t_{cloacal} and RR. The values in parentheses correspond to the standard deviations of each coefficient of adjustment.

$$t_{\text{surface}} = 10.95(1.206) + 0.704(0.029) \cdot t_{\text{db}} + 0.023(0.017) \cdot \text{RH} - 0.085(0.337) \cdot V \quad (\text{model 1})$$

$$t_{\text{surface}} = 11.998(0.837) + 0.704(0.029) \cdot t_{\text{bs}} \quad (\text{model 2})$$

$$t_{\text{surface}} = -8.651(1.725) + 0.536(0.023) \cdot \text{THI} \quad (\text{model 3})$$

$$t_{\text{surface}} = -8.843(1.750) + 0.532(0.023) \cdot \text{BGHI} \quad (\text{model 4})$$

$$t_{\text{surface}} = -21.062(2.432) + 0.110(0.005) \cdot \text{RHL} \quad (\text{model 5})$$

$$t_{\text{surface}} = 18.728(0.836) + 0.206(0.013) \cdot H \quad (\text{model 6})$$

$$t_{\text{surface}} = -104.504(29.603) + 3.392(0.737) \cdot t_{\text{cloacal}} \quad (\text{model 7})$$

$$t_{\text{surface}} = 28.282(0.572) + 0.060(0.008) \cdot \text{RR} \quad (\text{model 8})$$

Therefore, t_{surface} increases with increasing values of thermal-related variables, except for V (m s^{-1}). While the increase of t_{surface} indicates uncomfortable thermal conditions, the increase in V (m s^{-1}) can lead to higher heat dissipation through convection.

The increase in t_{surface} as the environment becomes warmer leads to a reduction in heat dissipation, with consequent heat stress symptoms (CURTO et al., 2007). Thus, the use of infrared thermography is essential to evaluate variations of surface temperature in birds.

Regarding the artificial intelligence-based models, we can observe that the fuzzy system resulted in the smallest errors (Table 2). Some fuzzy systems have better performances compared with ANNs (CALDEIRA et al., 2007; HERNÁNDEZ-JULIO et al., 2014; PONCIANO FERRAZ et al., 2014).

The system based on fuzzy rules, composed of four components – input variables, a set of linguistic rules, a fuzzy inference method and an output variable, generating a real number as an outcome (R CORE TEAM, 2015), is detailed in Table 3. The input variables for application of the fuzzy method in this study were t_{db} , RH and V. A set of 30 linguistic rules, was generated through the Mamdani's inference method, with surface temperature as the output variable. A weighting factor equal to 1 was attributed to each rule (CREMASCO et al., 2010; YANAGI JÚNIOR et al., 2012; SCHIASSI et al., 2014).

Mamdani's inference method proposes a binary fuzzy relation between two variables to model the set of rules. This method is based on the min-max inference composition rule,

involving inference rules of the type: If x is A and y is B then z is C (TANAKA, 1997; PEDRYCZ, GOMIDE, 1998).

Table 3. Composition of the rule system used in the fuzzy inference for the input variables: air dry-bulb temperature (t_{db}), air relative humidity (RH), air velocity (V), and output variable: surface temperature ($t_{surface}$).

RULES ¹	
1	If (t_{db} is very low) and (RH is low) and (V is low) Then ($t_{surface}$ is MF 1)
2	If (t_{db} is very low) and (RH is low) and (V is medium) Then ($t_{surface}$ is MF 1)
3	If (t_{db} is very low) and (RH is low) and (V is high) Then ($t_{surface}$ is MF 1)
4	If (t_{db} is very low) and (RH is high) and (V is low) Then ($t_{surface}$ is MF 3)
5	If (t_{db} is very low) and (RH is high) and (V is medium) Then ($t_{surface}$ is MF 4)
6	If (t_{db} is very low) and (RH is high) and (V is high) Then ($t_{surface}$ is MF 2)
7	If (t_{db} is low) and (RH is low) and (V is low) Then ($t_{surface}$ is MF 8)
8	If (t_{db} is low) and (RH is low) and (V is medium) Then ($t_{surface}$ is MF 7)
9	If (t_{db} is low) and (RH is low) and (V is high) Then ($t_{surface}$ is MF 8)
10	If (t_{db} is low) and (RH is high) and (V is low) Then ($t_{surface}$ is MF 6)
11	If (t_{db} is low) and (RH is high) and (V is medium) Then ($t_{surface}$ is MF 5)
12	If (t_{db} is low) and (RH is high) and (V is high) Then ($t_{surface}$ is MF 5)
13	If (t_{db} is medium) and (RH is low) and (V is low) Then ($t_{surface}$ is MF 10)
14	If (t_{db} is medium) and (RH is low) and (V is medium) Then ($t_{surface}$ is MF 10)
15	If (t_{db} is medium) and (RH is low) and (V is high) Then ($t_{surface}$ is MF 9)
16	If (t_{db} is medium) and (RH is high) and (V is low) Then ($t_{surface}$ is MF 13)
17	If (t_{db} is medium) and (RH is high) and (V is medium) Then ($t_{surface}$ is MF 11)
18	If (t_{db} is medium) and (RH is high) and (V is high) Then ($t_{surface}$ is MF 12)
19	If (t_{db} is high) and (RH is low) and (V is low) Then ($t_{surface}$ is MF 12)
20	If (t_{db} is high) and (RH is low) and (V is medium) Then ($t_{surface}$ is MF 14)
21	If (t_{db} is high) and (RH is low) and (V is high) Then ($t_{surface}$ is MF 12)
22	If (t_{db} is high) and (RH is high) and (V is low) Then ($t_{surface}$ is MF 18)
23	If (t_{db} is high) and (RH is high) and (V is medium) Then ($t_{surface}$ is MF 15)
24	If (t_{db} is high) and (RH is high) and (V is high) Then ($t_{surface}$ is MF 17)
25	If (t_{db} is very high) and (RH is low) and (V is low) Then ($t_{surface}$ is MF 19)
26	If (t_{db} is very high) and (RH is low) and (V is medium) Then ($t_{surface}$ is MF 17)
27	If (t_{db} is very high) and (RH is low) and (V is high) Then ($t_{surface}$ is MF 20)
28	If (t_{db} is very high) and (RH is high) and (V is low) Then ($t_{surface}$ is MF 16)
29	If (t_{db} is very high) and (RH is high) and (V is medium) Then ($t_{surface}$ is MF 19)
30	If (t_{db} is very high) and (RH is high) and (V is high) Then ($t_{surface}$ is MF 19)

¹MF: membership function

The building process of the fuzzy model is based on the steps of fuzzification (conversion of the input values into fuzzy values), inference (determination of the output values based on the pre-established rule systems) and defuzzification (conversion of the fuzzy values into numerical values).

Triangular, trapezoidal and Gaussian functions are generally used to characterize a fuzzy set represented by a membership function (ORTEGA, 2001). A linguistic variable is a variable whose values are names of fuzzy sets. Its primary function is to offer a systematic means for fair characterization of complex or ill-defined phenomena (GONÇALVES, 2007).

Triangular membership functions are shown in Figures 1 and 2. In Figure 1A, we can observe that the degrees of pertinence define the degree - between $[0;1]$ - to which a particular t_{db} value belongs to a fuzzy set (very low, low, medium, high, very high). The same structure is presented for the remaining input variables in Figures 1B (relative air humidity) and 1C (wind speed).

Input variables

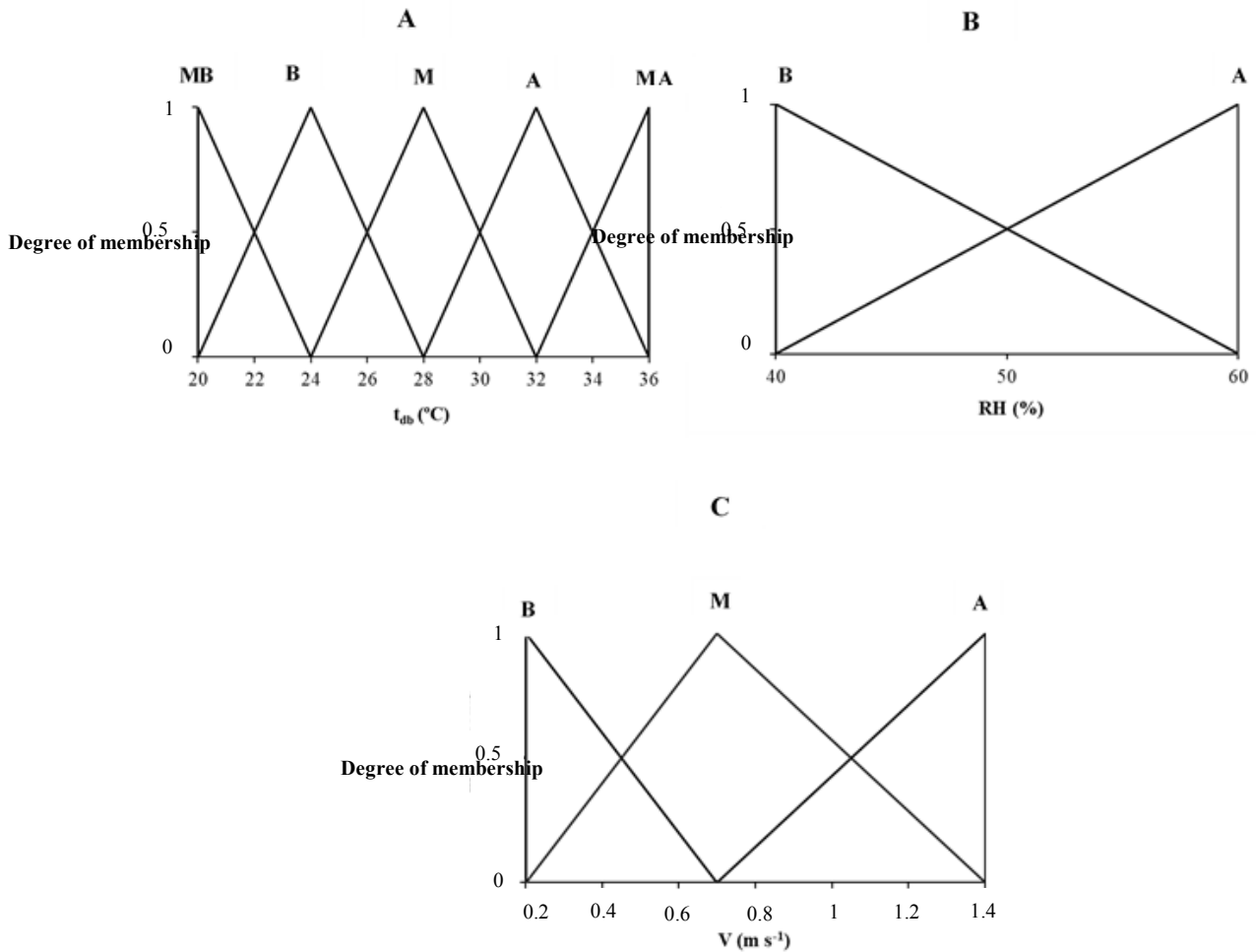


Figure 1. Membership function for the input variables: (A) air dry-bulb temperature (t_{db} , °C), (B) air relative humidity (RH, %) and (C) air velocity (V , $m s^{-1}$). VL: very low; L: low; M: medium; H: high; VH: very high.

The domain intervals for the input variables were: t_{db} [20; 36]; RH [40; 60]; V [0.2; 1.4] and for the output variable $t_{surface}$ the interval was [24.55; 37.35]. The set of rules was formed based on numeric data and consultation with experts. Following the combination of the set of rules, the fuzzy output sets are transformed into a precise system outcome. In such outcome, each membership function corresponds to a numerical value which depends on the information supplied to build the method (Figure 2).

Output variable

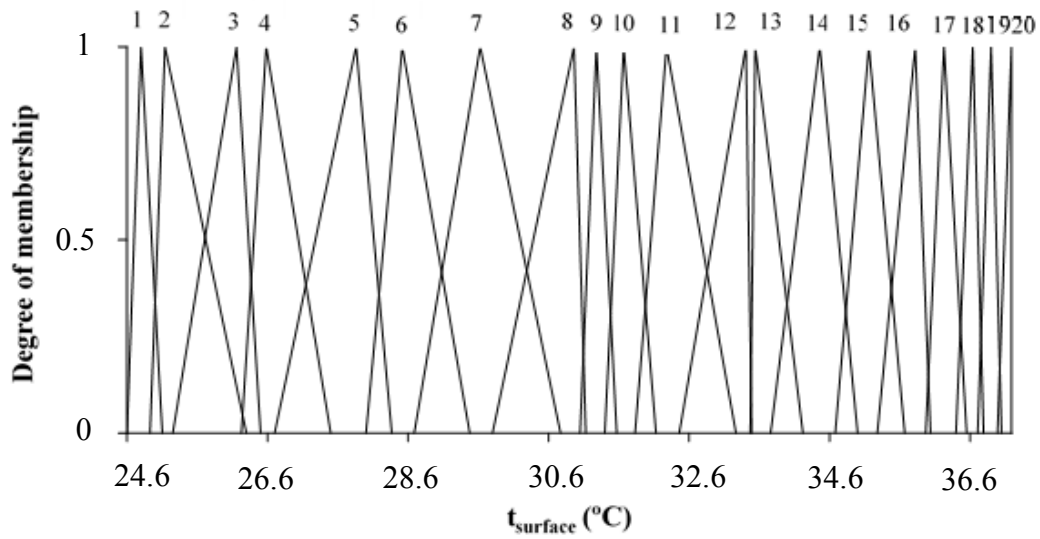


Figure 2. Membership function for the output variable surface temperature (t_{surface} , °C).

The graph showing the correlation between t_{surface} obtained experimentally and the response values predicted by the fuzzy system is shown in Figure 3, accompanied by their respective R^2 coefficient.

Based on the multivariate linear regression, the results indicate a coefficient of determination of 0.9983 for t_{surface} , which corroborates with the value of R^2 (0.994) found by Bahuti et al. (2019) when evaluating the body surface temperature of laying hens using the fuzzy method. The proposed fuzzy system predicts 99.83% of the interference of environmental variables (t_{db} , RH, and V), which compose the multivariate model on the birds' response t_{surface} .

HERNÁNDEZ-JULIO et al., 2020 when studying reproductive aspects of copepod crustaceans, applied the same methodology based on fuzzy sets using clusters and pivot tables and other methodologies such as ANNs and Adaptive Neuro-Fuzzy Inference System. Thus, they found the same significant configuration of the fuzzy methodology compared to the other applied methodologies, as well as in this work, where a better fit of the fuzzy systems (0.9983) was found compared to ANNs (0.9728) for the physiological variable t_{surface} .

The high correlation between the variables that characterize the thermal environment (t_{db} , RH, and V) and t_{surface} can be explained by the direct relationship between these variables and the physiology of the birds. Ruzal et al. (2011) state that air temperature (t_{db}) interferes with the redistribution of blood flow in the bird's body. Vasodilation of the blood vessels

occurs due to the need to dissipate body heat, which allows for an increase in blood flow and, consequently, maintenance of body temperature.

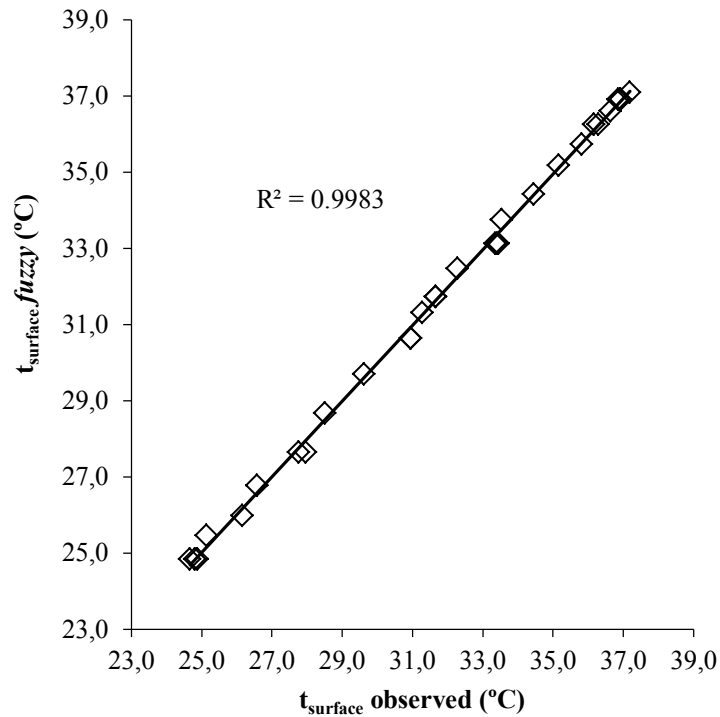


Figure 3. The functional relationship between the observed and fuzzy-predicted surface temperature of laying hens (t_{surface} , $^{\circ}\text{C}$).

The ANN methodology, whose purpose is to model complex mathematical functions (GRAVES, 2008), had an adjustment level for t_{surface} similar to that of the fuzzy method, with a value of 0.9728 for R^2 . This result, combined with those observed for other statistical indices (Figure 4), demonstrates the model's accuracy in describing the behavior of t_{surface} as a function of environmental-related variables in a more realistic approach.

Other studies highlighted the use of ANN as a high accuracy methodology with the potential to mitigate the impact of adverse physiological effects since it allows us to anticipate decision-making regarding the acclimatization systems. Carvalho (2017) developed controller-embedded software to control the thermal environment inside poultry buildings and evaluated the surface temperature of such birds, finding an R^2 value of 0.9118 in the validation step. This value is close to the observed in the present study. Nazareno et al. (2016) also structured an ANN to predict the mean surface temperature of poultry chicks and the microclimate during transportation; they obtained a value of 0.910 for R^2 .

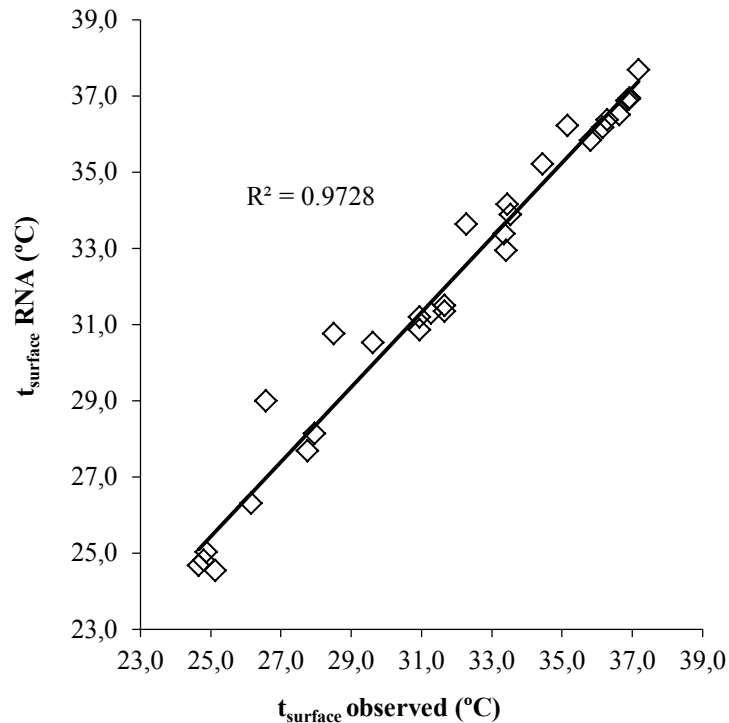
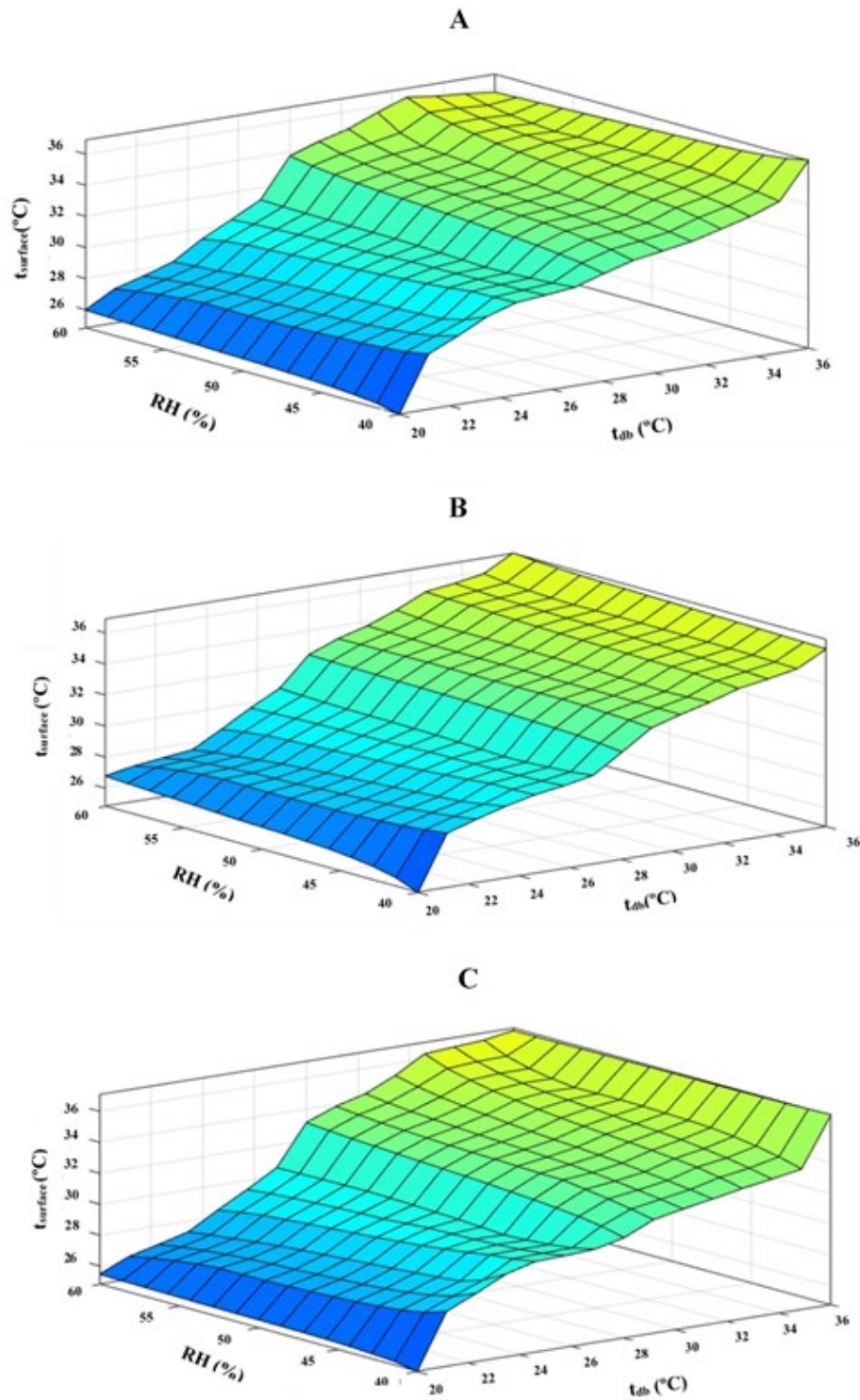


Figure 4. The functional relationship between the observed and ANN-predicted surface temperature of laying hens (t_{surface} , $^{\circ}\text{C}$).

Figures 5A, 5B and 5C show the behavior of t_{surface} simulated by the model of best performance – fuzzy – as a function of t_{db} and RH for V values of 0.2, 0.7, and 1.4 m s^{-1} , respectively. The t_{surface} increased with increasing t_{db} and RH. If the air surrounding the animal is hot, heating by convection is likely to occur (FERREIRA, 2016).

For a V value of 0.7 m s^{-1} (Figure 5B), we can verify a smoother mesh in scenarios in which t_{db} lies in the range between 22 and 28 $^{\circ}\text{C}$. Above 28 $^{\circ}\text{C}$, the increase in t_{surface} is sharper. This behavior demonstrates that increased ventilation rates lead to the activation of physiological mechanisms. According to Shinder et al. (2007), the vasomotor activity of the skin covered in feathers is minimal, and these areas can be characterized as heat-conserving regulators. Hence, the increase in ventilation helps dissipate body heat in birds.

As V increases to 1.4 m s^{-1} (Figure 5C), we can observe a higher capacity to maintain t_{surface} in lower values, even in hotter scenarios, rendering acclimatization easier. Castilho et al. (2015) claim that the amount of thermal energy stocked by a body mass unit determines the bird's body temperature, and such energy can be increased or decreased by thermogenesis and



thermolysis processes. In other words, the metabolism of birds adjusts itself depending on the intensity of the stressor.

Figure 5. The behavior of surface temperature of laying hens (t_{surface} , °C) as a function of air dry-bulb temperature (t_{db} , °C) and air relative humidity (RH, %) for air velocity (V) values of (A) 0.2 m s^{-1} , (B) 0.7 m s^{-1} and (C) 1.4 m s^{-1} .

Graphs A and B in Figure 6 show the behavior of t_{surface} as a function of t_{db} and V for RH values of 40% and 60%, respectively. In the present study, we observed that t_{surface} increases with increasing t_{db} , with higher values for environments with RH of 60% compared with those at 40%. As reported by Melo et al. (2016), the body parts which are covered in feathers favor thermal insulation and impose difficulties in heat exchange with the environment. Silva (2008) emphasizes that continuous heat exchange with the environment is of great importance for laying hens under conditions out of thermoneutrality; the activation of physiological adjustment as an attempt to maintain thermal balance is crucial.

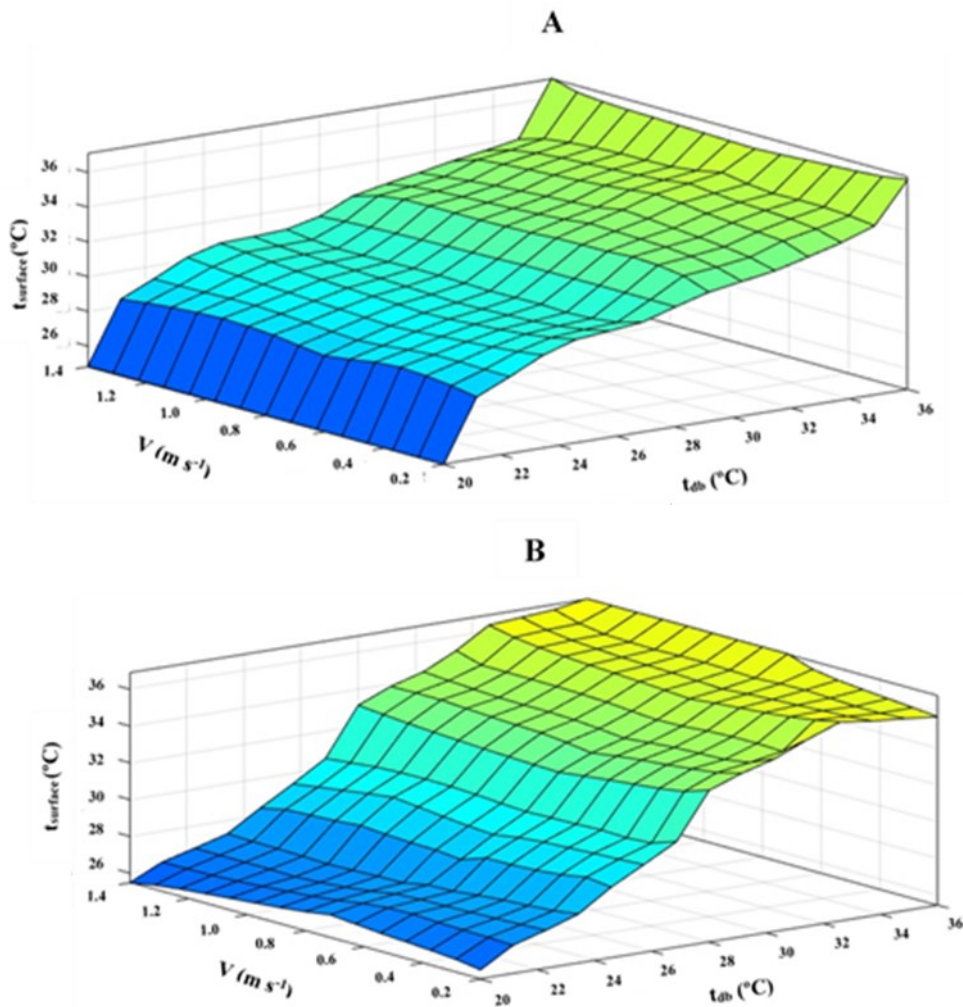


Figure 6. Behavior of surface temperature of laying hens (t_{surface} , °C) as a function of air dry-bulb temperature (t_{db} , °C) and air velocity (V m s^{-1}) for relative air humidity (RH, %) of (A) 40 and (B) 60%.

4. CONCLUSION

The main objective of this work stood out in the selection of the best practical model using different methodologies (empirical models and based on artificial intelligence) to control fans and nebulizers inside aviaries in order to predict a scenario of thermal comfort and discomfort in the poultry system of posture. Highlight for model 1, which is composed of variables t_{db} , RH and V, which have a direct effect on the configuration of the microclimate inside the commercial poultry buildings.

Animal thermal environment inside commercial poultry buildings can be monitored through surface temperature, a variable which rapidly responds to situations of thermal discomfort and changes in air temperature. Since the referred monitoring is a noninvasive technique, its use can become a strategy to control the microclimate inside buildings.

The artificial intelligence-based models had the best statistical indices among the models for prediction of the surface temperature of laying hens, being the fuzzy ($R^2 = 0.9983$) model was slightly superior to ANN ($R^2 = 0.9728$). The model adjusted as a function of air dry-bulb temperature was the empirical model with the best results. Therefore, such models can be used in the control of thermal environments inside commercial poultry buildings.

Acknowledgments

To Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), to Fundação de Amparo à Pesquisa do Estado de Minas Gerais (Fapemig), and to Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES), for financial support.

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FINAL CONSIDERATIONS

In laying hens, since welfare does not show a direct economic benefit, producers focus on animal productivity to predict comfort or discomfort. Currently, environmental issues, food security and animal welfare have been considered the three biggest challenges in the sector. In this context, there is a gradual increase in concern about the effect that the environment can have on the behavior and performance of animals.

Animal interaction and the environment must be considered when seeking greater productivity, and the different responses of the animal to the peculiarities of each region are decisive for poultry success. Thus, the identification of factors that influence the animal's life, such as thermal stress, caused by seasonal fluctuations in the environment, allows adjustments in management practices, making it possible to give them sustainability and economic viability.

The establishment of new thermal comfort limits obtained in this work, allows the management of the microclimate inside the facilities, as well as the updating of the limits reported in the literature. As well as, the application of computational and mathematical modeling techniques, they are tools that help in the prediction of the thermal comfort and discomfort of the birds, that is, in the evaluation of the thermal environment inside the production warehouses.

The results obtained in this work demonstrate that establishing thermal comfort limits according to the physiology of laying hens, and modeling the thermal environment according to the variables of the thermal environment, bioclimatic indexes, thermodynamic property and physiology of laying hens are strategies that impact directly and positively, within egg production systems.