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Thermal rearing environment effect on behavior and metabolic profile of laying hens

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Abstract: For the trial, 54 Bovans White laying hens were used at 95 weeks of age and were housed in groups of three birds per cage. A completely randomized design was used with a factorial scheme of treatments 2 × 3 (rations with two energy densities × three thermal environments). Corn-soybean meal based diets with similar composition, differing only in energy levels, were obtained with soybean oil inclusion (2750 kcal and 3250 kcal metabolizable energy (ME)). Climate-controlled rooms were used to mimic a thermoneutral environment (TE) (average temperature of 24.3 °C and relative humidity of 62.3%), a hot environment (HE) (average temperature of 30.2 °C and relative air humidity of 58.8%), and a cool environment (CE) (average temperature of 17.7 °C and relative humidity of 98.5%). The behavior (ethology and vocalization), physiological parameters (cloacal temperature and heat emission), and metabolic profile (serum biochemistry) of laying hens were analyzed. Laying hens' behavior was affected by levels of ME. With higher levels of ME, hens sat more frequently and spent more time eating when they received lower levels of ME in their diets. Laying hens under heat stress, ate, stopped, and walked more frequently than the group housed in a CE. The noise ratio (dB (A)) emitted by laying hens differed according to the thermal environment. Chickens reared in hot environments vocalized more than those raised in cool environments. Diets and thermal environments influence laying hen behaviors, with thermoneutral environments presenting greater comfort as evidenced by lower vocalization. Diets with 3250 kcal of ME present greater control of body heat reflected in lower production of adrenalin and lactate serum levels (g/L). A thermal environment above a TE is more stressful to laying hens than the CE; thus, a TE is recommended to improve laying hens' welfare.

Key words: Body temperature, **energy**, heat stress, lactate, vocalization

1. Introduction

The environmental temperature of hen houses is not effectively controlled, which influences the expression of laying hens' genetic potential of strains, performance, behavior, and welfare. Behavioral changes can be observed due to thermal stress, with changes in frequency of eating, drinking, and aggressive pecking [1]. Feed intake can decrease by 1.5% with the increase of each 1 °C [2]. Digestibility of nutrients is also negatively affected by thermal stress [3,4].

Decreases in feed intake and digestibility reduce energy ingestion, which is the main nutrient of hens' feed, and is necessary to maintain bodily functions such as movement, regulation of body temperature, tissue synthesis, and egg production [5].

Glucostatic control is a mechanism of ingestive behavior for hens, in which they consume feed primarily to satisfy their energy requirements. This theory can also explain the consumption behavior response of hens after a period of food deprivation: birds work harder for food the longer they have been without it [6–8].

Commercial laying hens are usually reared until the 70th week; however, when there is a higher supply of eggs on the market, high feed and chick prices, or delays in supply of 1-day-old chicks can extend the productive life until the 140th week and consequently the exposure time to thermally critical environments [9,10].

The present study aims to verify the effect of different levels of metabolized energy (ME) and thermal environments in behavioral, physiological, and metabolic profiles of laying hens at the end of laying.

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2. Materials and methods

This study was carried out according to the ethical principles and was approved by the local Research Ethics Committee (CEUA/UNIGRAN no. 009/16).

The assay was conducted in Dourados, State of Mato Grosso do Sul, Brazil (22.197157 Lat.; -54.938371 Log.) over 9 days; with five days for adaptation of birds to cages, diets, and air conditioning, and 4 days of data collection.

For the trial, 54 Bovans White laying hens were used at the end of the laying period, with an initial age of 95 weeks and an average weight of 1.726 ± 0.271 kg. During the period prior to testing, hens were given feed according to lineage manual [11] recommendations. The management and lighting program during the entire lifespan of these laying hens were based on Bovans White manual recommendations [1].

Cages were placed in acclimatized experimental rooms measuring 41 cm wide by 61 cm long and 41 cm high, equipped with a trough-type feeder and nipple-type drinker, and laying hens were housed in three cages for each treatment, divided into groups of three birds per cage. Therefore, laying hens were distributed in a factorial scheme of treatments with two ME levels and three thermal environments.

Diets were formulated according to [11] to achieve Bovans White Manual's recommendations and corn-soybean meal based on similar composition (Table 1), differing only in energy densities, obtained by including soybean oil (2,750 kcal of ME, and 3250 kcal of ME). Diets were supplied ad libitum.

Acclimatized rooms had controlled temperature and humidity to achieve the following conditions and the approximate Temperature-Humidity indexes (THI) [12] are shown:

Room 1: Thermoneutral environment (TE), with an average temperature of 24.3 °C and 62.3% relative humidity (THI: 71.1);

Room 2: Hot environment (HE), with an average temperature of 30.2 °C and 58.8% relative humidity (THI: 78.6);

Room 3: Cool environment (CE), with an average temperature of 17.7 °C and 98.5% relative humidity (THI: 63.4);

Data loggers (HOBO U14-001, Onset, USA) recorded temperature and humidity inside the acclimatized rooms, at a resolution of 0.1 °C (temperature) and 1% (humidity), and with an accuracy of ±0.5 °C (temperature) and ±1% (humidity). The air velocity was measured using an anemometer (Alnor, GGA-65P) and set to the same speed ($2m^3h^{-1}$) in the three acclimatized rooms.

Table 1. Composition of the experimental diets.

	Diets	
	ME levels (kcal/kg of feed)	
Ingredients	2750	3250
Maize meal	59.98	59.98
Soybean meal	23.43	23.43
Wheat meal	0.41	0.41
Dicalcium phosphate	1.06	1.06
Limestone	9.12	9.12
Salt	0.46	0.46
DL-methionine	0.16	0.16
Soybean oil	1.17	4.98
Inert	3.81	0
Premix (Vit. Min.)	0.4	0.4
	Calculated Composition, g/kg	
AME, kcal/kg	2750	3250
Crude protein, %	16.02	16.02
Ca %	3900	3900
P %	0.291	0.291
Na %	0.218	0.218
Met + Cys %	0.617	0.617
Lysine %	0.719	0.719
Methionine %	0.391	0.391
Threonine %	0.535	0.535
Linoleic acid	1.325	1.325

1Vitamin and mineral premix composition Fe, 0.04 g/kg; Cu, 10 mg/kg; Mg, 0.08 g/kg; Zn,0.1 g/kg; I, 0.832 mg/kg; Se, 3 mg/kg; retinyl acetate, 7000,00 UI/kg; cholecalciferol, 2500,00 UI/kg; α-tocopherol acetate, 8.00 UI/kg; menadione, 1.58 mg/kg; thiamine, 1.00 mg/kg; riboflavin, 4.00 mg/kg; Niacinn (minimum), 20.1 mg/kg; pantothenic acid (minimum), 7.22 mg/kg; pyridoxine, 1.00 mg/kg; folic acid, 0.296 mg/kg; Biotine, 0.02 mg/kg; cyanocobalamin, 9.6 mcg/kg; Choline (minimum), 0.3 g/kg; Methionine, 1.00 g/kg; Colistin, 7 mg/kg.

For behavior analysis, each room was equipped with a video camera (Mythos CCD Color model - 1.5mm lens) and connected to a computer for recording images during 1 h per day. Each laying hen was identified individually and observed for 10 min according to methodology adapted from [1], taking into account the following activities:

a) Eating;

b) Drinking water;

c) Panting—exhibiting rapid, shallow breathing with an open beak;

[1] Bovans. Nutrition Management Guide. Hendrix Genetics. [online] The Netherlands. Website https://www.bovans.com/en/product/bovans-white/2017 [accessed 25 july 2019]

d) Sitting—sitting on the floor of the cage;

e) Wing scattering—space could be seen between both wings and body;

f) Stopping (standing still)—not presenting any movement nor any behaviors;

g) Preening—trawling feathers with beak;

i) Pecking—pecking any object or parts of the cage.

j) Aggressive pecking—pecking any other bird aggressively.

k) Walking—when the bird moved inside the cage.

Vocalization was analyzed using the Sound Analyzer App, which captured the sound oscillations in decibels (dB (A)) for hundredths of seconds and generated a table for each vocalization control. Controls were recorded during the 4-day experimental period, one in the morning (8:00 a.m.), one in the middle of the day (1:00 p.m.) and one at the end of the day (5:30 p.m.), for a duration of 1 min per reading.

The physiological parameters were evaluated during the 4-day experiment, from 9:00 a.m. to 10:00 a.m., as follows:

1. Respiratory frequency (RR): thoracic movements hens performed in 1 min;

2. Cloacal temperature (CloT): measured with digital thermometer (Brasmed –32 to 45 °C) inserted in cloaca;

3. Body surface temperature (BT): measured with digital infrared thermometer (MINIPA-MT-320 –20 to 400 °C) with laser sight temperatures of the comb (TCb), claw (TCl), back (TB), and wing (TW). From these measurements body surface temperature was calculated using the formula adapted from [13]:

$BT = (0.06 \times TCb) + (0.7 \times TB) + (0.09 \times TCl) + (0.15 \times TW)$

After collecting physiological parameters, heat emissions were measured once a day throughout the entire experimental period, by cage and by one of the birds, using the Hotter HT3 thermographic camera, with a coefficient of emissivity of 0.96, and using the software IR Reporter V. 1.0, generating an average value of heat emission for each bird.

At the end of the experimental period, 5 mL blood samples were collected from each laying hen through cardiac puncture. Blood serum was processed in the semiautomated biochemical analyzer equipment (Sinnowa Model SX-3000m), using serological kits (Labtest kits®) following the protocols described by the manufacturer. Metabolic profile was determined by analyzing biochemical parameters glucose (GLU), triglyceride (TGL), cholesterol (COL), high density lipoprotein (HDL), low density lipoprotein (LDL), very low density lipoprotein (VLDL), total protein (TP), albumin (ALB), and lactate (LAC).

All data normalities of the residues and variances homogeneity were verified using the Shapiro–Wilk and Levene tests [14]. The vocalization data used the model $Y_{ijl} = \mu + A_i + T_j + T_j(A_i) + e_{ijl}$, and for the remaining data, the model $Y_{ijl} = \mu + A_i + D_j + D_j(A_i) + e_{ijl}$ was used, wherein: μ = overall average, A_i = effect of the environment, T_j = effect of time, $T_j(A_i)$ = effect of time and environment interaction, D_j = effect of diet, $D_j(A_i)$ = effect of diet and environment interaction, and e_{ijl} = error. The data were then submitted to ANOVA by the PROC MIXED of SAS 9.3. Means were compared using Tukey's test with a significance level of 5%.

3. Results

No interaction effect was observed among diets and environments for behavioral patterns in neither quantity nor time spent in each activity. However, laying hens sat more frequently when they were fed higher levels of ME. Time spent eating was higher for hens that received 2750 kcal ME diets.

Laying hens under heat stress ate, stopped, and walked more frequently than the group housed in a CE. Birds exposed to a TE were less active (Table 2). The environment also influenced the time that chickens spent preening, hens in the TE room spent more time preening than those in the other groups (Table 3). The noise ratio (dB(A)) emitted by laying hens differed according to the thermal environment during the four days of the experiment (Figure 1). Chickens reared in HEs vocalized more than those in a CE; while, among the three treatments, laying hens vocalized less in a TE. Another aspect observed was that the vocalization gradually reduced with the time of accommodation ($P = 0.001$), probably due to adaptation to the environment. Analyzing the incidence of vocalization during the day (Figure 2), the birds were noisier at the end of the day ($P = 0.001$), except those housed in a TE. Cloacal temperature was affected by diet; hens fed with 2750 kcal ME diets had higher values, especially in HEs (Table 4). The highest body temperatures and heat emissions observed in hens were in rooms with a HE, followed by a TE and, finally, a CE.

There was an interaction between environment and diet ($P = 0.011$) for the lactate concentration (g/L) of chickens' biochemical profile (Table 5), and the lactate was lower in birds exposed to the HE.

4. Discussion

Healthy animals use about 60% of the energy consumed to maintain basal metabolic rates. A reduction in energy availability interferes with their feed intake [15], which will in turn increase as a compensatory process, to the qualitative food restriction, reflecting the time and frequency chickens go through food intake and rest phases. Thus, hens eating higher energy density diets were satiated more quickly and were seated more often.

Table 2. Effect of thermal environment and metabolizable energy (in quantity of observations) on the behavioral patterns of laying hens.

Variables	Environment**						ASE*	P-value		
	Cool		Thermoneutral		Hot					
	Metabolizable energy***									
	2750 kcal	3250 kcal	2750 kcal	3250 kcal	2750 kcal	3250 kcal		Diet	Environment	Interaction
Eating	4.58^{A}	3.67^{A}	2.25^{C}	2.17^{C}	5.08^{B}	3.67^{B}	0.31	0.079	0.0004	0.479
Drinking	0.50	1.17	0.58	0.67	1.17	1.17	0.14	0.375	0.288	0.572
Panting	0	0	0	0	0	0	0.01	0.322	0.376	0.376
Sitting	0.42^{a}	0.42^{a}	0.25^{b}	0.75^{a}	0.25^{b}	1.92^{a}	0.19	0.038	0.227	0.131
Wing scattering	0.25	0	0.08	0.17	0.25	0.08	0.04	0.194	0.897	0.254
Stopped	5.42^{B}	6.75^{B}	3.83^{C}	4.92^{C}	6.83^{A}	7.83^{A}	0.41	0.060	0.0007	0.971
Preening	0.67	1.58	0.58	1.17	1.92	1.25	0.19	0.390	0.190	0.110
Pecking	2.50	2.25	0.67	2.17	3.67	4.33	0.49	0.501	0.096	0.760
Aggressive pecking	0.33	0.92	4.08	1.42	1.67	5.33	0.84	0.759	0.371	0.328
Walking	3.08^{B}	1.42^{B}	1.67^{A}	1.83^{A}	5.33^{C}	3.67^{C}	0.38	0.079	0.0009	0.348

*Average standard error; ** Different capital letters (A, B, C) in the same line indicate statistically significant difference regarding the effect of the environment according to the adjusted Tukey test ($P < 0.05$); *** Different lowercase letters (a, b) in the same line indicate statistically significant difference regarding the effect of the diet according to the adjusted Tukey test ($P < 0.05$).

Table 3. Effect of thermal environment and metabolizable energy in time (minutes) on the behavioral patterns of laying hens.

Variables	Environment**						ASE*	P-value		
	Cool		Thermoneutral		Hot					
	Metabolizable energy***									
	2750 kcal	3250 kcal	2750 kcal	3250 kcal	2750 kcal	3250 kcal		Diet	Environment	Interaction
Eating	296^{a}	204^{b}	215^{a}	155^{b}	262^{a}	111^{b}	19.65	0.016	0.338	0.647
Drinking	6	16	5	19	20	14	2.61	0.275	0.586	0.233
Panting	0	0	0	0	2	0	0.42	0.376	0.322	0.376
Sitting	57	39	48	114	110	109	16.97	0.633	0.317	0.552
Wing scattering	1	0	3	4	0	0	0.81	0.193	0.974	0.905
Stopping	214	237	257	232	162	309	18.69	0.242	0.927	0.217
Preening	7^{C}	21^{C}	24^{A}	57^{A}	19^{B}	11^{B}	4.93	0.162	0.033	0.209
Pecking	12	12	6	15	4	10	2.04	0.210	0.638	0.633
Aggressive pecking	0	2	7	2	2	9	1.56	0.712	0.569	0.288
Walking	24	24	18	19	20	20	2.75	0.960	0.444	0.995

*Average standard error; ** Different capital letters (A, B, C) in the same line indicate statistically significant difference regarding the effect of the environment according to the adjusted Tukey test ($P < 0.05$); *** Different lowercase letters (a, b) in the same line indicate statistically significant difference regarding the effect of the diet according to the adjusted Tukey Test ($P < 0.05$).

The ethological findings observed in the current study for layers housed in different environments and in the time in which they performed each action corroborates with [16], showing preference of birds for different environments and activities, as an indicative of well-being stages. According to these authors, high frequency

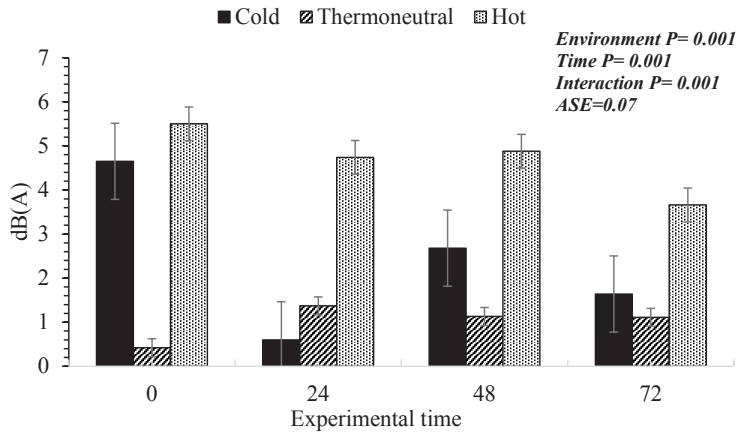

Figure 1. Birds vocalization in dB(A) emitted by birds in different thermal environments, during experimental time**.**

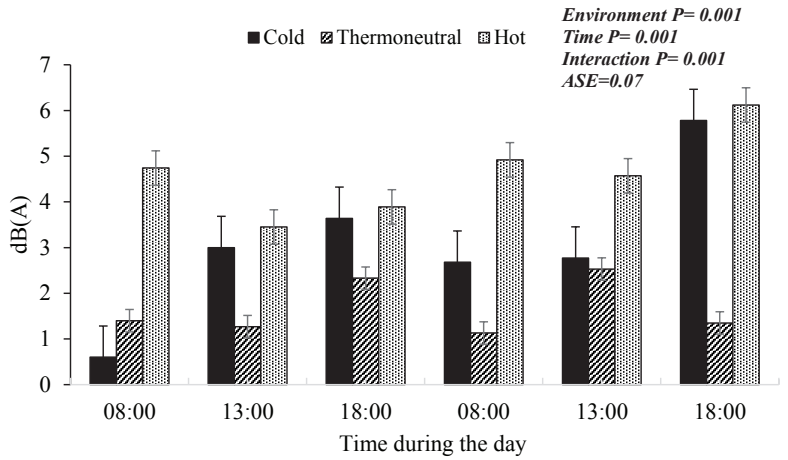

Figure 2. Birds vocalization in dB(A) emitted by birds in different thermal environments, at different hours of the day during experimental time.

of feeding, permanent alertness, and self-scratches are negatively associated with preference of a particular environment. Therefore, a TE is demonstrated to be better for laying hens' welfare.

The possibility of identifying welfare conditions using patterns of sound emitted by laying hens was demonstrated by [17]. The vocalizations emitted by laying hens have shown a specific profile when exposed to ambient thermal variations [18]. Amplitude and frequency of vocalizations were influenced by lower temperatures, and as a behavioral response, chickens formed groups and vocalization stabilized. Another aspect observed was that the hens gradually reduced their vocalization, probably due to their adaptation to the environment over the time of the experiment.

Chickens emit different sounds including "clucks" and "food calls," which are short, sharp, and tend to be produced in anticipation of rewards, indicating possible excitement, while "whines" and "gakel-calls" are more frequent in nonrewarding, frustrating contexts. At the end of the day, hens were fed in small amounts in feeders at specific times, which was possibly an additional stressor representing the absence of reward. Thus, "whines" and "gakel-calls" were emitted louder, demonstrating frustration. The present findings on the incidence of noisier vocalizations at the end of the day corroborate [19] the view that laying hens emit different types of calls that indicate the motivational state of these animals.

The maintenance of energy homeostasis is highly sophisticated, the sum of external and internal stimuli will influence nutrient intake, such as the genotype, diet composition and digestibility, feed form and processing, ME and aminoacid levels, and health status [20]. Lipids are recognized as a significant nutritional supplier of ME, since 1 g of lipids provides more energy than 1 g of carbohydrate (9 cal versus 4 cal). The "extra-caloric" effect

Table 4. Heat emission (°C) by the bird and physiological parameters: respiratory rate (RR), cloacal temperature (TCloacal), and body surface temperature (TBody) according to the diets and thermal environments.

Variables	Environment**						ASE*	P-value		
	Cool		Thermoneutral		Hot					
	Metabolizable energy***									
	2750 kcal	3250 kcal	2750 kcal	3250 kcal	2750 kcal	3250 kcal		Diet	Environment	Interaction
RR (mov/min)	21.7	19.5	19.5	22.5	18.5	21.5	0.63	0.337	0.809	0.180
TCloacal (°C)	40.6^{a}	40.1^{b}	40.7^{a}	40.5^{b}	41.0^{a}	40.3^{b}	0.12	0.047	0.518	0.653
TBody (°C)	28.8^{C}	28.1^{C}	29.1^{B}	29.4^{B}	31.8^{A}	31.6^{A}	0.36	0.652	0.0001	0.673
Heat emission (°C)	21.2^{C}	21.9^{C}	28.6^{B}	28.3^{B}	32.2^{A}	31.3^{A}	0.70	0.716	0.0001	0.597

*Average standard error; ** Different capital letters (A, B, C) in the same line indicate statistically significant difference regarding the effect of the environment according to the adjusted Tukey test ($P < 0.05$); *** Different lowercase letters (a, b) in the same line indicate statistically significant difference regarding the effect of the diet according to the adjusted Tukey test ($P < 0.05$).

Table 5. Biochemical parameters: glucose (GLI), triglycerides (TGL), cholesterol (COL), high-density lipoprotein (HDL), low-density lipoprotein (LDL), very-low-density lipoprotein (VLDL), total protein (TP), albumin (ALB), and lactate (LAC) according to the diets and thermal environments.

Variables	Environment**						ASE*	P-value		
	Cool		Thermoneutral		Hot					
	Metabolizable energy***									
	2750 kcal	3250 kcal	2750 kcal	3250 kcal	2750 kcal	3250 kcal		Diet	Environment	Interaction
GLI (mg/dL)	217.67	195.67	237.33	222.67	212.33	207.33	32.20	0.299	0.308	0.862
TGL (mg/dL)	363.00	374.50	276.33	234.00	205.67	331.00	6.74	0.662	0.403	0.603
COL (mg/dL)	159.67	215.00	114.33	112.00	109.67	188.67	17.26	0.225	0.262	0.633
HDL (mg/dL)	2.00	6.33	1.50	6.66	2.50	5.67	1.20	0.161	0.999	0.955
LDL (mg/dL)	56.40	146.60	43.90	62.70	81.70	116.80	16.77	0.228	0.507	0.712
VLDL mg/dL)	72.60	74.90	55.27	46.80	41.13	66.20	6.44	0.662	0.403	0.603
PT (g/L)	12.53	10.98	11.32	8.87	10.63	11.78	0.52	0.339	0.377	0.309
ALB (g/L)	3.02	2.58	2.29	2.63	2.22	2.73	0.41	0.653	0.584	0.409
LAC (g/L)	6.80^{Aa}	7.20^{Aa}	8.50^{Aa}	5.70^{Ab}	4.80^{Ba}	5.60^{Ba}	0.38	0.158	0.008	0.011

*Average standard error; ** Different capital letters (A, B, C) in the same line indicate statistically significant difference regarding the effect of the environment according to the adjusted Tukey test ($P < 0.05$); *** Different lowercase letters (a, b) in the same line indicate statistically significant difference regarding the effect of the diet according to the adjusted Tukey test ($P < 0.05$).

can be attributed to lipids, comparatively, due to the lower metabolic heat increasingly reached by digestive and absorptive metabolism [21].

Lipid digestion and absorption are affected by a chicken's age [22], with older ones using lipids more efficiently. The higher the energy concentration in the diet, the lower the amount of consumed feed. Consequently, metabolic heat production will be lower, which reflects in lower cloacal temperature.

Environmental temperature affects consumption in an inversely proportional way [3]. The expression of the hypothalamic mRNA of the gonadotropin-inhibiting hormone (GnIH) is altered in heat stress conditions, inhibiting anorexigenic neuropeptide, which is an appetite stimulant, reducing feed intake and increasing body heat.

Results obtained in the present study coincide with this physiological process; environments affect the body temperature and the heat emission, and the highest

temperatures were observed in hens housed in a HE, followed by a TE and lastly, a CE. Also, feed intake was inhibited due to reduction in the expression of the gonadotropin-inhibitory hormone (GnIH) [23].

Lactate analysis is widely used in veterinary science for determination of tissue hypoxia and resistance to anaerobiosis; however, hyperlactatemia is an effect of higher levels of adrenaline, stimulating sarcolemmal activity of the Na^+/K^+-ATPase, and the anaerobic glycolysis [24]. Therefore, the increase in serum lactate of laying hens in HEs indicates increases in adrenaline production due to thermal stress and restlessness.

Diets and thermal environments influence laying hens' behaviors. Particularly, tested TEs present greater comfort and welfare, as evidenced by lower vocalization. Diets with 3250 kcal of ME present greater control of body heat, reflected in lower production of adrenalin and lactate serum levels (g/L). Thermal environments above thermoneutrality are more stressful to laying hens than CEs; thus, a TE is recommended to improve laying hens' welfare.

Conflict of interest

The authors report no conflicts of interest.

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