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Identical thermal stress coupled with different temperature and humidity combinations affects nutrient digestibility and gut metabolites of laying hens

Da-Hye Kim[1], Yoo-Bhin Kim[1], Sang Hyeok Lee[1], Yoo-Kyung Lee[2], Sung-Dae Lee[2], Kyung-Woo Lee[1]*

[1] Konkuk University, Department of Animal Science and Technology, Gwangjin-gu, Seoul, Republic of Korea.
[2] Rural Development of Administration, National Institute of Animal Science, Wanju-gun, Jeollabuk-do, Republic of Korea.

***Corresponding author:**
kyungwoolee@konkuk.ac.kr

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ABSTRACT - The present study investigated whether the same temperature-humidity index (THI) values under different conditions of air temperature and relative humidity (RH) would affect the thermoregulatory, nutritional, and behavioral responses of laying hens. One hundred twenty Hy-Line Brown laying hens (60-weeks-old) were divided equally in two environmental chambers: 26 °C with 70% RH (hRH75) and 30 °C with 30% RH (hT75) for 28 days. The two ambient environments (hRH75 and hT75) had an identical THI value of 75, calculated using an empirical formula for laying hens. Neither hRH75 nor hT75 affected rectal and body-surface temperatures and heart and respiratory rates. The concentration of volatile fatty acids in fecal excreta were altered by the thermal treatments. hT75 vs. hRH75 decreased the proportion of acetate and increased the proportion of propionate in fecal samples. hT75 vs. hRH75 lowered the digestibility of dry matter, crude protein, and neutral detergent fiber at 14 days. Thermal treatments did not affect heat stress-associated behavioral responses including feeding, drinking, panting, and wing elevation at any stage. Laying hens exposed to the same THI at different temperatures and RH exhibit equal physiological responses including rectal and body-surface temperatures, heart and respiratory rates, and behavioral responses. Nonetheless, high-temperature treatment (hT75; 30 °C and 30% RH) vs. low temperature treatment (hRH75; 26 °C and 70% RH) affects nutrient digestibility and gut metabolites, suggesting that there are negligible but discernable responses to temperature in the gut physiology.

Keywords: behavior, heat stress, physiology, temperature-humidity index

1. Introduction

Stress is a biological adaptive response that restores homeostasis and alters the normal physiological state of animals. Heat stress usually occurs in animals when there is an imbalance resulting in the generation of more heat than its dissipation (Renaudeau et al., 2012). The heat stress caused by environmental factors such as temperature, relative humidity (RH), and thermal irradiation (Akbarian et al., 2016) is considered a major challenge affecting the global poultry industry. It deteriorates egg production (Ebeid et al., 2012), antioxidant parameters (Ibtisham et al., 2019; Khan et al., 2023), and nutrient digestibility (Kim et al., 2020) and alters behavioral patterns (Mack et al., 2013). Laying hens are particularly vulnerable to heat stress because of their extended long production cycle (up to 74 weeks of age), lack of sweat glands, feathered bodies, and inherently high metabolic rates (Narinç et al., 2016).

The temperature-humidity index (THI) is an indicator of thermal stress and has been used to predict the impact of the thermal environment on the thermoregulatory status of animals (Zulovich and DeShazer, 1990; Tao and Xin, 2003). The THI chart was developed using an empirical equation factoring in temperature and RH that determine the quantitative and qualitative aspects of heat stress in animals (Zulovich and DeShazer, 1990). The THI for laying chickens is defined by the equation:

$$THI = 0.6T_{db} + 0.4T_{wb} \quad (1)$$

in which T_{db} = dry-bulb temperature (°F) and T_{wb} = wet-bulb temperature (°F) (Zulovich and DeShazer, 1990). The THI chart has been classified into four stress zones—comfort zone (THI < 70), alert zone (THI 70–75), danger zone (THI 76–81), and emergency zone (THI > 81)—depending on the severity of heat stress imposed. This chart implies that laying hens would receive identical heat stress intensity from the environment if they were exposed to high temperatures with low humidity, or low temperatures with high humidity. There is a lack of literature on the effects of equal THI values coupled with different combinations of temperature and RH on the nutrition, physiology, and behavior of laying hens. It is hypothesized that equal THI values result in the same thermoregulatory responses, but with different effects on the other evaluated responses. Previously, we reported that high temperature deteriorates the stress indicators including performance, physiology, and behavior of laying hens (Kim et al., 2020, Kim et al., 2021a) and that thermal environments (equal THI of 75) with different combinations of temperature and humidity do not affect laying production and stress indicators including corticosterone in yolk, albumen, and plasma samples in laying hens (Kim et al., 2021b). The present study was conducted to monitor the thermoregulatory responses (i.e., nutrition, physiology, and behavior indices) of laying hens exposed to the same THI values.

2. Material and Methods

2.1. Ethical matters

Research on animals was conducted according to ethical guidelines provided by the Institutional Committee on Animal Use (KU19008).

2.2. Experiment design and treatments

The experiment was conducted on a farm in Chungju, Chungbuk-do, South Korea (36°38' N, 127°29' E). One hundred twenty 60-week-old laying hens (Hy-Line Brown) were housed in two identical environmental chambers. Each chamber had one tier of 20 cages, placed 1 m above the ground. The cage dimensions were 41 × 37 × 40 cm (length × width × height), with each cage containing water nipples and a trough feeder and equipped with a heater (MCP-300; MAXCON Co., Bucheon, Korea), an air-conditioner (AR07J5174HA, SAMSUNG, Suwon, Korea), a humidifier (MH-601A; mtechwin Co., Gimhae, Korea), a dehumidifier (NED-062P; NAWOOEL Co., Gimpo, Korea), and a main controller. There were three hens per cage. Two cages were considered a replicate and each hen was provided with 506 cm^2 of floor space. Hens were initially adapted to the chambers for two weeks prior to the experiment, at an ambient temperature of 24±1.5 °C with 50±3.0% (THI of 70) RH and a 16L:8D photoperiod. At the end of the adaptation period, the ambient temperature in the first chamber was adjusted to 26±1.2 °C with 70±2.2% RH (hRH75), whereas the second chamber was adjusted to 30±1.0 °C and 30±2.5% RH (hT75) for 28 days. The air-conditioner and the heater were used to maintain the indicated temperature while the humidifier and the dehumidifier were employed to maintain the indicated relative humidity in the chambers. Temperature and humidity data were recorded at 10 min intervals during the experimental period. Both thermal treatments had a THI of 75. The THI was calculated using the formula (equation 1) proposed by Zulovich and DeShazer (1990). Temperature and humidity loggers (MHT-381SD; Lutron Electronic Enterprise Co., Taipei, Taiwan) were placed in each chamber. Corn-soybean meal-based commercial laying hen diets were used (Table 1). Laying hens had *ad libitum* access to clean water and feed throughout the experimental period.

Table 1 - Ingredients and chemical composition of the basal diet

Ingredient	%
Corn	43.0
Wheat	5.59
Soybean meal (45% CP)	5.14
Dehulled rice	4.0
Rice bran	2.0
Corn germ meal	5.52
Rapeseed meal	3.0
Dried distillers grains with solubles	17.0
Liquid condensed molasses solubles	1.0
Liquid choline	0.06
Limestone	10.7
Monodicalcium phosphate	0.66
Salt	0.22
Carrier (corn)	1.25
Methionine - 100%	0.06
Lysine sulfate - 54%	0.25
Tryptophan	0.30
Mineral mix[1]	0.12
Vitamin mix[2]	0.14
Total	100.00
Calculated or analyzed chemical composition	
Nitrogen-corrected apparent metabolizable energy[3] (kcal/kg)	2,600
Dry matter[4]	89.2
Crude protein[4]	14.8
Calcium[4]	5.15
Total phosphorus[4]	0.60
Available phosphorus[3]	0.28
Salt[3]	0.15
Lysine[3]	0.64
Methionine[3]	0.32
Methionine + Cysteine[3]	0.60
Threonine[3]	0.52
Tryptophan[3]	0.16

[1] Vitamin mixture provided the following nutrients per kg of diet: vitamin A, 4.6 mg; vitamin D_3, 0.08 mg; vitamin E, 14 mg; vitamin K_3, 1.4 mg; vitamin B_1, 1.12 mg; vitamin B_2, 2.8 mg; vitamin B_6, 3.92 mg; vitamin B_{12}, 0.014 mg; niacin, 56 mg; pantothenic acid, 5.6 mg; folic acid, 0.28 mg; biotin, 0.14 mg; choline, 260.4 mg.
[2] Mineral mixture provided the following nutrients per kg of diet: Mn, 70 mg; Zn, 50 mg; Fe, 50 mg; Cu, 7 mg; I, 0.75 mg; Co, 0.4 mg; Se, 0.17 mg.
[3] Calculated values.
[4] Analyzed values.

2.3. Measurements of rectal temperature, body-surface temperature, heart rate, and respiratory rate

On days 7, 14, 21, and 28, following thermal treatment, one hen per cage was randomly selected, and rectal temperature was measured by inserting a rectal thermometer to a depth of 3 cm into the rectum. Body-surface temperature was recorded at three different sites (i.e., head, chest, and leg) using a thermal imaging camera (Cat® S60: equipped with a FLIR™ Lepton, FLIR Systems Inc., Wilsonville, OR, USA) as previously described by Cangar et al. (2008) and Jeong et al. (2020). In brief, the birds were picked up and placed on the floor, and the images were taken at a distance of about 30 cm as fast as possible to reduce stress effects. The birds were handled by wearing latex gloves to avoid influences of heat and moisture of the hands on the temperature of the feathers. The heart rate (beats per minute) was determined in one chicken per replicate using a stethoscope placed on the right

side of the breast, and the respiratory rate (breaths per minute) was measured by counting the number of breaths of each chosen chicken; a stopwatch was used to time 1 min for both measurements.

2.4. Fecal nutrient digestibility

During the last three days of weeks 2 and 4, the weighed quantities of feed provided and fresh fecal excretion samples per replicate were quantitatively collected to determine the apparent total tract digestibility of dry matter (DM), crude protein (CP), crude fat, neutral detergent fiber (NDF), and crude ash. Excreta from each replicate were collected four times daily, stored at −20 °C, and pooled per replicate. The excreta samples were dried in a drying oven at 55 °C for 72 h and ground for chemical analysis. Feed and excreta samples were analyzed in duplicate for DM (Method 930.15; AOAC, 2005), crude fat (Method 920.39; AOAC, 2005), CP (Method 990.03; AOAC, 2005), NDF (Goering and Van Soest, 1970), and crude ash (Method 942.05; AOAC, 2005).

2.5. Fecal volatile fatty acid analysis

Fresh excreta samples freed from uric acid and cecal droppings were collected fortnightly and processed to measure the volatile fatty acids (VFA) contents on the same day of sampling. Approximately 1 g of excreta was added to 9 mL of cold distilled water and homogenized using an Ultra Turrax homogenizer (Digital Ultra-Turrax T25, IKA, Staufen, Germany), and 0.05 mL of saturated $HgCl_2$, 1 mL of 25% H_3PO_4, and 0.2 mL of 2% pivalic acid were added to the mixture and centrifuged at 1,000 × *g* at 4 °C for 20 min. The supernatant (1.5 mL) was then collected and stored at −20 °C for later analysis. The concentrations of VFA in the samples were measured using gas chromatography (6890 Series GC System, HP, Palo Alto, CA, USA) as previously described by Kim et al. (2018).

2.6. Assessment of behavioral response

Behavioral observations were performed on days 7, 14, 21, and 28. High-definition video cameras (HDR-XR160, Sony Corp., Tokyo, Japan) were set up to record the behavioral responses of the hens (6 replicates per treatment). Behavioral responses were observed using 10 min video sampling from 20:00 to 22:00 h (before the lights were turned off). Behavioral responses were recorded according to a predefined ethogram (Table 2) and were presented following a previously published method (Mack et al., 2013; Kim et al., 2021a).

Table 2 - Behavioral ethogram of laying hens

Behavioral response	Definition
Posture	
Standing	Both feet are in contact with the cage floor; no other body part is in contact with cage floor.
Sitting	Most of the ventral region of the hen's body is in contact with cage floor. No space is visible between the cage floor and the hen.
Action	
Feeding	Head is located inside feeder.
Drinking	Beak is in contact with a nipple drinker.
Preening	Gently pecking or scratching its own feathers.
Aggression pecking	Beak contacts another hen.
Wing position	
Elevated	A space can be seen between hen's wings and body.
Not elevated	There is no observable space between the hen's wings and body.
Respiration	
Panting	Beak is open and respiration rate is abnormally rapid.
Not panting	Beak is closed, and respiration rate is normal.

2.7. Statistical analysis

Two adjacent cages were used as experimental units. The results are presented as least square means and standard deviation. All data were analyzed using the paired t-test procedure in SAS (Statistical Analysis System, version 9.4). The following mathematical model was adopted:

$$Y_{ij} = \mu + \alpha_i + \varepsilon_{ij}$$

in which Y_{ij} = dependent variable, μ = mean of the variable, α_i = effect of treatments, and ε_{ij} = random error. The observed behavioral data collected weekly over 28 days were combined for statistical analysis. For all analyses, $P<0.05$ and $0.05<P<0.10$ were considered as statistical significance and tendency.

3. Results

Laying hens exposed to either treatments, hRH75 or hT75 (THI = 75), exhibited no differences in rectal temperature and heart and respiratory rates (Table 3). Hens in both treatments hRH75 and hT75 exhibited no changes in body-surface temperatures at any stage (Table 4), although hRH75-exposed hens had slightly higher (P = 0.060) chest temperatures than hT75-exposed hens on day 7.

Acetate was proportionally the most dominant VFA on days 14 and 28 (Table 5). Interestingly, total VFA ranged from 36.3 to 45.9 mmol/g feces on day 14, and 78.9 to 91.0 mmol/g feces on day 28 (data not shown). The proportion of acetate in fecal samples was higher in hRH75-exposed hens than in hT75-exposed hens (P = 0.061). In contrast, treatments hRH75 vs. hT75 lowered the proportion of propionate in fresh fecal samples (P = 0.046).

Table 3 - Effect of different ambient temperatures with an equal temperature-humidity index (THI = 75) on rectal temperature (°C), heart rate (beat/min), and respiratory rate (breath/min)[1]

	Thermal treatment[2]				P-value
	hRH75		hT75		
	Mean	SD	Mean	SD	
Rectal temperature					
Day 7	41.11	0.448	41.26	0.389	0.435
Day 14	40.92	0.204	41.05	0.227	0.195
Day 21	41.16	0.151	41.10	0.082	0.283
Day 28	41.18	0.132	41.17	0.142	0.872
Heart rate					
Day 7	377.4	25.05	363.6	26.68	0.249
Day 14	302.1	18.71	312.3	25.04	0.316
Day 21	321.0	36.82	309.6	27.16	0.441
Day 28	291.0	14.76	298.2	24.17	0.432
Respiratory rate					
Day 7	28.80	3.795	27.60	6.450	0.618
Day 14	25.20	3.795	29.10	10.397	0.280
Day 21	27.00	5.099	28.80	4.733	0.424
Day 28	24.60	3.406	25.20	3.795	0.714

SD - standard deviation.
[1] n = 10 replicates per treatment.
[2] hRH75: temperature = 26 °C and relative humidity = 70%; hT75: temperature = 30 °C and relative humidity = 30%.

Table 4 - Effect of different ambient temperatures with an equal temperature-humidity index (THI = 75) on body-surface temperature (°C)[1]

	Thermal treatment[2]				P-value
	hRH75		hT75		
	Mean	SD	Mean	SD	
Day 7					
Head	30.50	2.071	30.60	1.883	0.915
Chest	26.90	2.421	25.00	1.710	0.060
Leg	26.70	2.539	26.80	2.364	0.926
Day 14					
Head	31.80	0.595	31.90	0.934	0.806
Chest	25.92	1.542	26.97	1.141	0.101
Leg	28.81	1.665	29.41	2.140	0.493
Day 21					
Head	32.03	1.266	32.27	1.506	0.704
Chest	28.34	1.234	27.74	2.704	0.531
Leg	27.59	2.323	26.69	2.979	0.461
Day 28					
Head	31.70	1.284	32.20	1.503	0.434
Chest	25.68	1.130	25.74	1.546	0.922
Leg	27.57	2.512	27.76	1.981	0.853

SD - standard deviation.
[1] n = 10 replicates per treatment.
[2] hRH75: temperature = 26 °C and relative humidity = 70%; hT75: temperature = 30 °C and relative humidity = 30%.

Table 5 - Effect of different ambient temperatures with an equal temperature-humidity index (THI = 75) on concentration of fecal volatile fatty acids[1] (VFA; % of total VFA[2])

	Thermal treatment[3]				P-value
	hRH75		hT75		
	Mean	SD	Mean	SD	
Day 14					
Acetate	83.30	9.068	75.07	4.35	0.061
Propionate	10.09	4.339	14.55	2.47	0.046
Butyrate	9.071	3.598	10.37	2.73	0.490
Day 28					
Acetate	88.14	3.785	89.16	3.703	0.577
Propionate	5.548	2.543	5.300	2.130	0.829
Butyrate	6.311	2.075	5.544	1.796	0.421

SD - standard deviation.
[1] n = 10 replicates per treatment.
[2] Total VFA = acetate + propionate + butyrate.
[3] hRH75: temperature = 26 °C and relative humidity = 70%; hT75: temperature = 30 °C and relative humidity = 30%.

Treatments hT75 vs. hRH75 lowered the digestibility of DM (P = 0.023), CP (P = 0.010), and NDF (P = 0.052) (Table 6). However, the digestibility of crude fat and crude ash were not affected by the thermal treatments by day 14. At 28 days, none of the thermal treatments affected nutrient digestibility, although treatments hT75 vs. hRH75 tended to decrease the digestibility of CP (P = 0.114) and crude ash (P = 0.072) by approximately 10.5%.

Table 6 - Effect of different ambient temperatures with an equal temperature-humidity index (THI = 75) on nutrient digestibility[1]

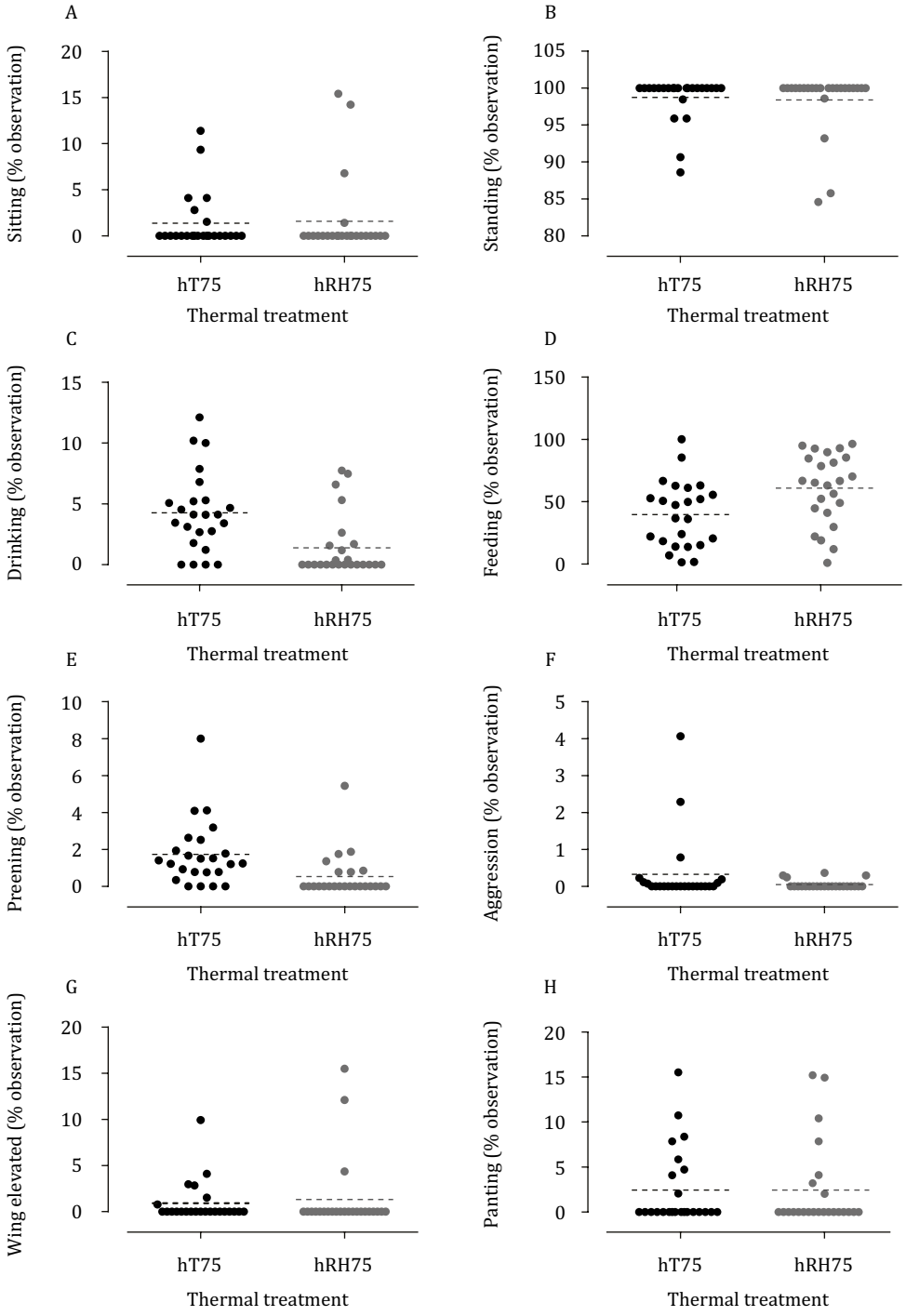
	Thermal treatment[2]				P-value
	hRH75		hT75		
	Mean	SD	Mean	SD	
Day 14					
Dry matter (%)	79.54	3.87	74.96	4.33	0.023
Crude protein (%)	77.71	6.40	63.79	13.76	0.010
Crude fat (%)	92.99	1.57	92.03	4.00	0.489
Neutral detergent fiber (%)	56.39	8.27	47.64	10.42	0.052
Ash (%)	50.06	7.79	43.97	12.90	0.217
Day 28					
Dry matter (%)	76.38	2.25	73.09	8.31	0.242
Crude protein (%)	72.39	7.81	65.14	11.39	0.114
Crude fat (%)	93.62	1.26	93.36	2.83	0.791
Neutral detergent fiber (%)	56.01	2.08	48.26	17.17	0.173
Ash (%)	75.95	4.30	67.91	11.88	0.072

SD - standard deviation.
[1] n = 10 replicates per treatment.
[2] hRH75: temperature = 26 °C and relative humidity = 70%; hT75: temperature = 30 °C and relative humidity = 30%.

As an indicator of heat stress for laying hens (Kim et al., 2021a), behavioral patterns were measured using video recordings on days 7, 14, 21, and 28 (Figure 1A-1H). None of the thermal treatments affected the behavioral patterns (data not shown). Thus, the behavioral patterns obtained at the four time points were pooled per replicate to simplify presentation. When all the observations monitored during the experimental period were pooled, three behavioral patterns (i.e., drinking, feeding, and preening) tended to be altered by thermal treatments—hT75 vs. hRH75 increased drinking and preening and decreased feeding.

4. Discussion

In a previous study, we observed that exposure to high temperatures lowered egg production, impaired gut functions, increased stress indicators including an increase of corticosterone in plasma and eggs, heterophil to lymphocyte ratio, altered heat-stress associated behaviors, and elevated physiological responses such as body temperature and respiratory rate (Kim et al., 2020, 2021a). In a subsequent study (Kim et al., 2021b), we reported that laying hens raised in the equal thermal environment (THI = 75) with different combinations of temperature and RH (30 °C and 30% RH; 26 °C and 70% RH) exhibited no differences in egg production and stress physiology (i.e., blood heterophil to lymphocyte ratio and corticosterone levels in plasma, and egg yolk and albumen). Here, we explored the effects of equal thermal environments (THI = 75) combined with different temperatures and RH on cardiac and digestive physiology and behavioral patterns of laying hens. As previously observed, the equal thermal environment (THI = 75) coupled with different combinations of temperature and RH (i.e., hRH75 or hT75) did not affect the rectal and body temperature, cardiac physiology including heart and respiratory rate, and behavioral patterns. Thus, our results confirmed that the THI chart can predict the thermal stress of laying hens (Zulovich and DeShazer, 1990; Purswell et al., 2012). However, we found that equal thermal treatments affected the indicators of gut physiology (i.e., gut metabolites and nutrient digestibility). In both thermal treatments (hT75 vs. hRT75), acetate levels decreased, but propionate levels increased in fresh fecal samples at day 14. Additionally, in both chambers, the digestibility of nutrients in the hens decreased by day 14 of heat treatment. Taken together, our study provides evidence that equal thermal treatments (THI = 75) with different combinations of temperature and



RH - relative humidity.
hRH75: temperature = 26 °C and RH = 70%; hT75: temperature = 30 °C and RH = 30%.
Values are least squares means.
Dots represent the 24 pooled observations per treatment.

Figure 1 - Effect of heat stress on behavioral responses.

RH had identical effects on the behavior and cardiac physiology, but exhibited an unequal effect on the gut physiology of laying hens.

In the chambers, we regulated temperature (26 °C vs. 30 °C) and RH (70% vs. 30%) to create thermal alert zone (THI = 75) using equation 1 generated for laying hens (Zulovich and DeShazer, 1990). It could be argued that the lack of cardiac and behavioral responses in this study might be

attributed to the difference in environmental temperature being only 4 °C (THI = 75; from 26 vs. 30 °C). Yahav (2000) reported that temperature, not RH, is the dominant driving factor of heat stress affecting the laying performance and physiology of laying hens. However, Cai et al. (2020) and Kim et al. (2020) found that air temperature differing by 5 or 6 °C caused a reduction in laying production and nutrient digestibility, increase in body-surface temperature, and alterations in behavioral patterns of laying hens. Thus, if temperature is considered to be the single determinant of heat stress for laying hens, the parameters for cardiac and digestive functions, and behavioral patterns could have been detected. Therefore, the lack of physiological and behavioral responses is not because of the temperature between the hT75 and hRT75 treatments, but because both thermal treatments were maintained at the same THI value. In future experiments, a clear explanation could be given if two environmental chambers with conditions of 24 °C with 95% RH and 32 °C with 15% RH, respectively (THI = 75), could be constructed, but this is difficult to achieve in practical experimental settings.

It is well documented that high environmental temperatures induce a cascade of physiological and metabolic changes (i.e., elevated rectal and body-surface temperatures) in laying hens (Mashaly et al., 2004; Rozenboim et al., 2007; Xie et al., 2015; Chang et al., 2018; Barrett et el., 2019; Xing et al., 2019; Kim et al., 2021a). Additionally, the heart rate of chickens elevates to increase blood flow to the capillary vessels to dissipate the body-generated sensible heat (Yahav, 2009). Finally, increasing their respiratory rate is the main mechanism used by poultry to dissipate latent heat (Sahin et al., 2009). All cardiac functions described as the indicators of heat stress were not altered between the two thermal treatments in this study; this supports our view that the THI values can be accepted as reliable indicators of heat stress in laying hens.

Extended heat exposure is a major environmental stressor that has a negative effect on nutrient digestibility (He et al., 2018). We observed that the digestibility of DM, CP, and NDF were significantly impaired in laying hens exposed to treatments hT75 vs. hRH75, and this effect was noted at 14 days, but not at 28 days. It is not yet clear whether the observed reduction in nutrient digestibility by hT75 vs. hRH75 can be attributed to hT75-induced inhibition in either the digestion or absorption processes, or both. Khan et al. (2012) reported that heat stress could inhibit the activity of digestive enzymes. In addition, it has been reported that heat stress inhibits the absorption of nutrients in the small intestine (Bonnet et al., 1997; Zhang et al., 2017). Thus, it is likely that digestion and nutrient absorption processes in the gastrointestinal tract may be more affected by treatment hT75 than by hRH75, although both had an equal THI value.

It can be postulated that impaired CP and NDF digestibility by hT75 vs. hRH75 noted in this study could have increased the substrate for gut microbiota and led to the altered VFA concentrations. As previously observed by Kim et al. (2020), heat stress lowered the digestibility of NDF but increased the VFA concentration in fecal droppings of laying hens. Similarly, Wang et al. (2019) observed elevated concentration of acetate, propionate, and butyrate in heat stressed laying hens exposed to an environmental temperature of 38 °C compared with those exposed to 28 °C. In this study, we observed that the equal thermal treatments affected the proportion of acetate and propionate, which indicated a close link between the thermal treatments and gut physiology. In contrast, hT75 vs. hRH75 did not increase the percentage of VFA equally. Therefore, the hT75-mediated increase in substrates for bacterial fermentation cannot be fully responsible for the observed VFA percentage. However, a clear explanation for this was not obtained and needs to be addressed. Further studies are necessary to investigate the concentration of digesta in the ileum or ceca and gut microbiome profiles, which can help clarify the observed effects on gut physiology. Our study tentatively suggests that the equal thermal environments (THI = 75) combined with different temperature and RH had an impact on gut microbiome and metabolites in laying hens and manifested predominantly as changes in fecal VFA concentration.

Many researchers have suggested that walking and standing behavioral responses and feed intake are reduced during heat stress conditions, while behavioral responses such as sitting, drinking, wing elevation, and panting are increased to dissipate excess heat (Gowe and Fairfull, 2008; Lara and

Rostagno, 2013; Mack et al., 2013; Sohail et al., 2013; Rostagno, 2020). In this study, no differences in behavioral responses were observed between the thermal treatments, confirming that equal THI had no effect on heat stress-associated behaviors. Interestingly, when each recorded behavioral pattern was pooled, hT75 vs. hRH75 increased drinking but decreased feeding behavior, indicating that temperature might affect the behavior of chickens in a discernible manner under chronic heat stress experimental settings.

5. Conclusions

The results of this study support the conclusion that the equal thermal environments (THI = 75), created with different temperature-RH combinations, do not affect behaviors and cardiac physiology, but altered the gut physiology of laying hens.

Conflict of Interest

The authors declare no conflict of interest.

Author Contributions

Conceptualization: D.-H. Kim, Y.-K. Lee, S.-D. Lee and K.-W. Lee. Data curation: D.-H. Kim, Y.-B. Kim, Y.-K. Lee, S.-D. Lee and K.-W. Lee. Formal analysis: D.-H. Kim, S.H. Lee and K.-W. Lee. Funding acquisition: K.-W. Lee. Investigation: D.-H. Kim, Y.-B. Kim and S.H. Lee. Methodology: D.-H. Kim. Project administration: K.-W. Lee. Resources: K.-W. Lee. Supervision: K.-W. Lee. Writing – original draft: D.-H. Kim. Writing – review & editing: D.-H. Kim and K.-W. Lee.

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References

Akbarian, A.; Michiels, J.; Degroote, J.; Majdeddin, M.; Golian, A. and De Smet, S. 2016. Association between heat stress and oxidative stress in poultry; mitochondrial dysfunction and dietary interventions with phytochemicals. Journal of Animal Science and Biotechnology 7:37. https://doi.org/10.1186/s40104-016-0097-5

AOAC - Association of Official Analytical Chemists. 2005. Official methods of analysis of the AOAC International. 18th ed. AOAC International, Gaithersburg, MD.

Barrett, N. W.; Rowland, K.; Schmidt, C. J.; Lamont, S. J.; Rothschild, M. F.; Ashwell, C. M. and Persia, M. E. 2019. Effects of acute and chronic heat stress on the performance, egg quality, body temperature, and blood gas parameters of laying hens. Poultry Science 98:6684-6692. https://doi.org/10.3382/ps/pez541

Bonnet, S.; Geraert, P. A.; Lessire, M.; Carre, B. and Guillaumin, S. 1997. Effect of high ambient temperature on feed digestibility in broilers. Poultry Science 76:857-863. https://doi.org/10.1093/ps/76.6.857

Cai, X.; Zhang, L.; Chen, X.; Zhang, H.; Xue, H.; Lu, Y.; Tang, J. and Lu, Y. 2020. *Orthosiphon stamineus* and rosmarinic acid reduce heat stress in laying hens. Livestock Science 240:104124. https://doi.org/10.1016/j.livsci.2020.104124

Cangar, Ö.; Aerts, J.-M.; Buyse, J. and Berckmans, D. 2008. Quantification of the spatial distribution of surface temperatures of broilers. Poultry Science 87:2493-2499. https://doi.org/10.3382/ps.2007-00326

Chang, Y.; Wang, X. J.; Feng, J. H.; Zhang, M. H.; Diao, H. J.; Zhang, S. S.; Peng, Q. Q.; Zhou, Y.; Li, M. and Li, X. 2018. Real-time variations in body temperature of laying hens with increasing ambient temperature at different relative humidity levels. Poultry Science 97:3119-3125. https://doi.org/10.3382/ps/pey184

Ebeid, T. A.; Suzuki, T. and Sugiyama, T. 2012. High ambient temperature influences eggshell quality and calbindin-D28k localization of eggshell gland and all intestinal segments of laying hens. Poultry Science 91:2282-2287. https://doi.org/10.3382/ps.2011-01898

Goering, H. K. and Van Soest, P. J. 1970. Forage fiber analysis (apparatus, reagents, procedures, and some applications). Agriculture Handbook No. 379. Washington, DC.

Gowe, R. S. and Fairfull, R. W. 2008. Breeding for resistance to heat stress. p.13-29. In: Poultry production in hot climates. 2nd ed. Daghir, N. J., ed. CABI, Wallingford, UK.

He, S. P.; Arowolo, M. A.; Medrano, R. F.; Li, S.; Yu, Q. F.; Chen, J. Y. and He, J. H. 2018. Impact of heat stress and nutritional interventions on poultry production. World's Poultry Science Journal 74:647-664. https://doi.org/10.1017/S0043933918000727

Ibtisham, F.; Nawab, A.; Niu, Y.; Wang, Z.; Wu, J.; Xiao, M. and An, L. 2019. The effect of ginger powder and Chinese herbal medicine on production performance, serum metabolites and antioxidant status of laying hens under heat-stress condition. Journal of Thermal Biology 81:20-24. https://doi.org/10.1016/j.jtherbio.2019.02.002

Jeong, S. B.; Kim, Y. B.; Lee, J. W.; Kim, D. H.; Moon, B. H.; Chang, H. H.; Choi, Y. H. and Lee, K. W. 2020. Role of dietary gamma-aminobutyric acid in broiler chickens raised under high stocking density. Animal Nutrition 6:293-304. https://doi.org/10.1016/j.aninu.2020.03.008

Khan, R. U.; Naz, S.; Nikousefat, Z.; Selvaggi, M.; Laudadio, V. and Tufarelli, V. 2012. Effect of ascorbic acid in heat-stressed poultry. World's Poultry Science Journal 68:477-490. https://doi.org/10.1017/S004393391200058X

Khan, R. U.; Naz, S.; Ullah, H.; Ullah, Q.; Laudadio, V.; Qudratullah; Bozzo, G. and Tufarelli, V. 2023. Physiological dynamics in broiler chickens under heat stress and possible mitigation strategies. Animal Biotechnology 34:438-447. https://doi.org/10.1080/10495398.2021.1972005

Kim, D. H.; Han, S. M.; Keum, M. C.; Lee, S.; An, B. K.; Lee, S. R. and Lee, K. W. 2018. Evaluation of bee venom as a novel feed additive in fast-growing broilers. British Poultry Science 59:435-442. https://doi.org/10.1080/00071668.2018.1476675

Kim, D. H.; Lee, Y. K.; Lee, S. D.; Kim, S. H.; Lee, S. R.; Lee, H. G. and Lee, K. W. 2020. Changes in production parameters, egg qualities, fecal volatile fatty acids, nutrient digestibility, and plasma parameters in laying hens exposed to ambient temperature. Frontiers in Veterinary Science 7:412. https://doi.org/10.3389/fvets.2020.00412

Kim, D. H.; Lee, Y. K.; Lee, S. D.; Kim, S. H. and Lee, K. W. 2021a. Physiological and behavioral responses of laying hens exposed to long-term high temperature. Journal of Thermal Biology 99:103017. https://doi.org/10.1016/j.jtherbio.2021.103017

Kim, D. H.; Lee, Y. K.; Kim, S. H. and Lee, K. W. 2021b. The impact of temperature and humidity on the performance and physiology of laying hens. Animals 11:56. https://doi.org/10.3390/ani11010056

Lara, L. J. and Rostagno, M. H. 2013. Impact of heat stress on poultry production. Animals 3:356-369. https://doi.org/10.3390/ani3020356

Mack, L. A.; Felver-Gant, J. N.; Dennis, R. L. and Cheng, H. W. 2013. Genetic variations alter production and behavioral responses following heat stress in 2 strains of laying hens. Poultry Science 92:285-294. https://doi.org/10.3382/ps.2012-02589

Mashaly, M. M.; Hendricks 3rd, G. L.; Kalama, M. A.; Gehad, A. E.; Abbas, A. O. and Patterson, P. H. 2004. Effect of heat stress on production parameters and immune responses of commercial laying hens. Poultry Science 83:889-894. https://doi.org/10.1093/ps/83.6.889

Narinç, D.; Erdoğan, S.; Tahtabiçen, E. and Aksoy, T. 2016. Effects of thermal manipulations during embryogenesis of broiler chickens on developmental stability, hatchability and chick quality. Animal 10:1328-1335. https://doi.org/10.1017/S1751731116000276

Purswell, J. L.; Dozier III, W. A.; Olanrewaju, H. A.; Davis, J. D.; Xin, H. and Gates, R. S. 2012. Effect of temperature-humidity index on live performance in broiler chickens grown from 49 to 63 days of age. In: Proceedings of the IX International Livestock Environment Symposium (ILES IX). American Society of Agricultural and Biological Engineers, St. Joseph.

Renaudeau, D.; Collin, A.; Yahav, S.; Basilio, V.; Gourdine, J. L. and Collier, R. J. 2012. Adaptation to hot climate and strategies to alleviate heat stress in livestock production. Animal 6:707-728. https://doi.org/10.1017/S1751731111002448

Rostagno, M. H. 2020. Effects of heat stress on the gut health of poultry. Journal of Animal Science 98:skaa090. https://doi.org/10.1093/jas/skaa090

Rozenboim, I.; Tako, E.; Gal-Garber, O.; Proudman, J. A. and Uni, Z. 2007. The effect of heat stress on ovarian function of laying hens. Poultry Science 86:1760-1765. https://doi.org/10.1093/ps/86.8.1760

Sahin, K.; Sahin, N.; Kucuk, O.; Hayirli, A. and Prasad, A. S. 2009. Role of dietary zinc in heat-stressed poultry: a review. Poultry Science 88:2176-2183. https://doi.org/10.3382/ps.2008-00560

Sohail, M. U.; Ijaz, A.; Younus, M.; Shabbir, M. Z.; Kamran, Z.; Ahmad, S.; Anwar, H.; Yousaf, M. S.; Ashraf, K.; Shahzad, A. H. and Rehman, H. 2013. Effect of supplementation of mannan oligosaccharide and probiotic on growth performance, relative weights of viscera, and population of selected intestinal bacteria in cyclic heat-stressed broilers. Journal of Applied Poultry Research 22:485-491. https://doi.org/10.3382/japr.2012-00682

Tao, X. and Xin, H. 2003. Acute synergistic effects of air temperature, humidity, and velocity on homeostasis of market–size broilers. Transactions of the ASAE 46:491-497. https://doi.org/10.13031/2013.12971

Wang, S.; Mahfuz, S. and Song, H. 2019. Effects of flammulinavelutipes stem base on microflora and volatile fatty acids in caecum of growing layers under heat stress condition. Revista Brasileira de Ciência Avícola 21:eRBCA-2019-0989. https://doi.org/10.1590/1806-9061-2019-0989

Xie, J.; Tang, L.; Lu, L.; Zhang, L.; Lin, X.; Liu, H. C.; Odle, J. and Luo, X. 2015. Effects of acute and chronic heat stress on plasma metabolites, hormones and oxidant status in restrictedly fed broiler breeders. Poultry Science 94:1635-1644. https://doi.org/10.3382/ps/pev105

Xing, S.; Wang, X.; Diao, H.; Zhang, M.; Zhou, Y. and Feng, J. 2019. Changes in the cecal microbiota of laying hens during heat stress is mainly associated with reduced feed intake. Poultry Science 98:5257-5264. https://doi.org/10.3382/ps/pez440

Yahav, S. 2000. Relative humidity at moderate ambient temperatures: its effect on male broiler chickens and turkeys. British Poultry Science 41:94-100. https://doi.org/10.1080/00071660086475

Yahav, S. 2009. Alleviating heat stress in domestic fowl: different strategies. World's Poultry Science Journal 65:719-732. https://doi.org/10.1017/S004393390900049X

Zhang, P.; Yan, T.; Wang, X.; Kuang, S.; Xiao, Y.; Lu, W. and Bi, D. 2017. Probiotic mixture ameliorates heat stress of laying hens by enhancing intestinal barrier function and improving gut microbiota. Italian Journal of Animal Science 16:292-300. https://doi.org/10.1080/1828051X.2016.1264261

Zulovich, J. M. and DeShazer, J. A. 1990. Estimating egg production declines at high environmental temperatures and humidities. ASAE Paper No. 90-4021.