 doi:10.20944/preprints202011.0251.v1

High temperature with low humidity or *vice versa* does not affect performance and physiology of laying hens

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Simple summary

The present study was investigated to test the hypothesis the practical relevance of temperature-humidity index (THI) to predict the production and physiology of laying hens. Two environment conditions employing high temperature with low relative humidity (HL75) or vice versa (LH75) were made but both had equal THI values (being THI 75) indicating equal thermal stress for laying hens. Neither LH75 nor HL75 affected laying performance

 doi:10.20944/preprints202011.0251.v1

including egg production, egg weight, egg mass, feed intake, and feed conversion ratio, plasma biochemical parameters, and stress indicators. Our study suggests that laying hens that are exposed to equal THI values will receive similar thermal stress. The results of this study will be served as a scientific basis for management decisions and handling laying hens under thermally challenging conditions.

Abstract: The present study investigated the effect of different ambient temperature and relative humidity (RH) but equal temperature-humidity index (THI) on laying performance, egg quality, heterophil to lymphocyte ratio (H/L ratio), corticosterone (CORT) concentration in blood, yolk and albumen, and plasma biochemical parameters in laying hens. One hundred and twenty commercial hens (Hy-Line Brown) aged 60 weeks were allocated into 2 environmental chambers. Laying hens were subjected to either one of two thermal treatments, i.e., 26ºC and 70% RH (LH75) and 30ºC and 30% RH (HL75) for 28 days. Both thermal treatments had equal THI being 75. Neither LH75 nor HL75 affected ($P > 0.05$) laying performance including egg production, egg weight, egg mass, feed intake, and feed conversion ratio. Plasma biochemical parameters such as total cholesterol, high-density lipoprotein cholesterol, triglyceride, calcium, magnesium, and phosphorus was not altered ($P > 0.05$) by thermal treatments. As to the stress indicators, both environment regimes failed ($P > 0.05$) to affect blood H/L ratio and CORT levels in plasma, yolk and albumen although albumen CORT levels were elevated ($P < 0.05$) in LH75 vs. HL75 at days 3, 7, and 28. In conclusion, our study suggests that laying hens performed and responded equally when they were exposed to equal THI environment conditioned from either 26ºC and 70% RH or 30ºC

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and 30% RH. The results of this study will be served as a scientific basis for management decisions and handling under thermally challenging conditions.

Keywords: heat stress, temperature humidity index, laying performance, egg quality, laying hens, stress indicators

1. Introduction

The optimum temperature for performance/thermoneutral zone is between 19 to 22°C for laying hens [1]. Laying hens exposed ambient temperature above thermoneutral zone experiences heat stress that leads to physiological defense mechanisms against heat challenge [2]. In addition, it has been reported that heat stress per se affects egg production [3], egg quality [4], and physiological [5,6] and stress responses [7] of laying hens.

Temperature-humidity index (THI) as the heat or stress index has been used to assess the impact of the thermal environment on thermoregulatory status of animals and used to prevent temperature stress [8,9]. Both ambient temperature and relative humidity (RH) are used as the variables to calculate THI values [8-12] and temperature vs. humidity is considered being more contributing to THI values. Zulovich and DeShazer [8] developed the THI chart for laying hens based on egg production levels and physiological responses. The THI chart has been classified into four stress levels ranging from comport (THI < 72), alert (THI 70–75), danger (THI 76–81), and emergency (THI > 81) zones. This chart can be used to assess the stress levels loaded on the laying hens subjected to each THI zones.

Considering the lack of sweat glands and heavily reliance on panting for thermoregulation in chickens, the THI can be ideally used to assess the performance and physiological response

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of laying hens. Theoretically, the same THI values calculated with different combinations of temperature and RH will expose the laying hens to the equal thermal (i.e., heat) stress. Thus, we hypothesized that equal THI values with different combinations of ambient temperature and RH would equally affect laying hens with regards to laying performance, egg quality and stress indicators (i.e., heterophil to lymphocyte [H/L] ratio and corticosterone [CORT]). The present study was set up to test our hypothesis in laying hens.

2. Material and Methods

2.1 Animal care

All hens used in this experiment were cared following the protocols approved by the Institutional Animal Care and Use Committee at Konkuk university (KU19008).

2.2 Birds and Experiment Design

One hundred twenty 60-week-old laying hens (Hy-Line Brown) were housed in two environmental chambers. Each chamber equipped with a heater, an air-conditioner, a humidifier, a de-humidifier, and a main controller, and had one-tier 20 cages, 1 m high from the floor. Each cage with 41 cm × 37 cm × 40 cm (length × depth × height) had nipples and a trough feeder and 3 hens per cage were housed. Two cages were considered a replicate and each hen was provided 506 cm^2 floor space. Hens were initially adapted to the chambers for 2 weeks at ambient temperature of 24℃ with RH of 50% and lighting program of 16L/8D. At the end of adaptation period, the first chamber received ambient temperature of 26ºC with 70% RH (LH75) while the second chamber exposed to ambient temperature of 30ºC with 30% RH (HL75) for the period of 28 days. Both thermal treatments had equal THI being 75. The THI was calculated per the formula of Zulovich and DeShazer, [8]. THI layers = 0.6 Tdb

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+ 0.4 Twb where THI = temperature-humidity index in °F, Tdb = dry-bulb temperature in °F, and Twb = wet-bulb temperature in °F. Temperature and humidity loggers (MHT-381SD; Lutron Electronic Enterprise Co., Taipei, Taiwan) were placed in each chamber. A corn-soybean meal-based commercial layer diets were used (Table 1). Laying hens had ad libitum access to water and feed throughout the experimental period.

2.3 Egg production and quality

Eggs laid was collected daily at 9:00 and weighed per replicate. Hen-day egg production was calculated as total eggs laid/hen-days multiplied by 100. Feed conversion ratio was expressed as kg of feed consumed per kg of egg produced or kg of feed consumed per 12 eggs laid. Eggs (n=6/replicate) were collected 3 consecutive days at the beginning and on a weekly basis to measure egg qualities. Specific gravity of the eggs was determined using the saline floatation method [13] using 6 salt solutions (1.100, 1.090, 1.080, 1.070, 1.060, and 1.050) at room temperature. Haugh unit, eggshell strength, eggshell thickness, and yolk color were tested by the digital egg tester (DET-6000, Navel, Kyoto, Japan). The residual albumen adhering to the eggshells was removed using an absorbent paper and dried in room temperature to determine eggshell weight.

2.4 Measurement of CORT and H/L in blood

At days 0, 2, 3, 7, 8, 12, 14, 16, 21, and 28 following temperature treatment, ten birds per treatment (n=10/treatment) were randomly selected for blood collection. Blood was drawn from wing vein into the heparinized tube and special care was paid not to sample same hens within three weeks. One drop of whole blood was smeared on the slide glass and dyed using the Differential Quik Stain Kit (Polysciences Asia-Pacific, Inc., Taipei, Taiwan), and

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heterophils and lymphocytes (for H/L ratio) were counted under the light microscope (Olympus BX 43, Olympus Optical Co. Ltd., Tokyo, Japan). Plasma was separated by centrifugation at 200 g for 15 min and stored at -20°C until the analysis. Plasma CORT concentrations were determined by CORT ELISA kit (Enzo life science Inc, ADI-901-097, Farmingdale, NY, USA) per the manufacturer's instructions. In addition, plasma samples collected at days 0, 3, 7, 14, 21, and 28 were analyzed for total cholesterol, triglyceride, high-density lipoprotein cholesterol, calcium, magnesium, and phosphorus using an automatic blood chemical analyzer (Film DRI CHEM 7000i, Fuji film, Tokyo, Japan). Nitric oxide in plasma samples was measured using modified Griess reagent (Sigma-Aldrich, St. Louis, MO, USA) as described elsewhere [14].

2.5 CORT in egg yolk and albumen

Three eggs per replicate were collected for determination of CORT in egg yolks and albumens at days 0, 2, 3, 7, 8, 12, 14, 16, 21, and 28 following temperature treatment. They were cracked open, yolk and albumen were separated, and pooled. The pooled yolk and albumen per replicate were homogenized and used to measure CORT concentration with CORT ELISA kit (Enzo life science Inc, ADI-901-097, Farmingdale, NY, USA) as described elsewhere [15,16].

2.6 Statistical analysis

Two adjacent cages considered an experimental unit. The results were presented as least square means and pooled standard error of the mean. All data were analyzed using the paired t-test procedure of SAS (SAS Institute Inc, Cary, NC, USA). Correlation coefficients were estimated between stress indicators (i.e., CORT in egg yolk and plasma, and blood H/L ratio)

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by the correlation (CORR) procedure of SAS (SAS Inst. Inc., Cary, NC, USA). All α values less than 0.05 were considered significant.

3. Result

Body weight (kg) at the beginning and the end of the experiment ranged 1.94 to 1.97. Laying performance was not altered on a weekly basis between treatments and thus presented for the whole period (Table 2). When hens were exposed to THI 75 conditioned with either LH75 or HL75, no differences were noted in all measured variables including hen-day egg production, egg weight, egg mass, and feed conversion ratio. Both LH75 and HL75 failed to affect weekly egg qualities including Haugh unit, eggshell strength, eggshell thickness, specific gravity, and absolute and relative weight of eggshell (Figure 1).

Neither LH75 nor HL75 regime affected plasma biochemical profiles including total cholesterol, high-density lipoprotein cholesterol, triglycerides, calcium, phosphorus, and magnesium (Figure 2). Nitric oxide, an indicator of innate immunity, was not altered by temperature/humidity regimes (i.e., LH75 or HL75).

We measured multiple stress indicators to assess the stress response of laying hens exposed to either LH75 or HL75. As response of laying hens to ambient temperature might be altered or variable during the course of heat exposure, we assayed the H/L ratio in blood, and CORT in plasma and eggs at several time points following heat treatment. As shown in Table 3, H/L ratio ranged ($P > 0.05$) 0.155 to 0.351 for hens exposed to LH75 and 0.163 to 0.329 for those to HL75. In addition, plasma CORT levels were not altered ($P > 0.05$) in laying hens between treatments. Plasma CORT in LH75-exposed hens fell within 8.01 to 14.47 ng/ml and those on HL75 regime had 8.62 to 14.12 ng CORT/ml.

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CORT can be deposited in both yolk and albumen during the formation of eggs which can be used to assess the status of stress in laying hens [17]. The CORT concentrations in yolk and albumen were presented in Table 4. Two THI-75 regimes conditioned with LH75 and HL75 failed to affect CORT levels deposited in yolk. The CORT levels ranged from 9.03 to 16.13 ng per g wet-yolk for LH75-conditioned regime, and from 9.05 to 16.18 ng per g wet-yolk for HL75-conditioned regime. Both temperature/RH conditions did not affect albumen CORT levels except for albumen collected from days 3, 7, and 28 (Table 4). Hens exposed to LH75 vs. HL75 had elevated CORT levels in albumin sampled at day 3 ($P = 0.096$), day 7 ($P = 0.003$), and day 28 ($P = 0.084$). Correlation analysis revealed small to moderate association between plasma CORT and yolk CORT ($r = 0.288$, Figure 3A), yolk CORT and albumen CORT ($r=0.499$, Figure 3B), and CORT in plasma and albumen ($r = 0.207$, Figure 3F). Negligible associations between H/L ratio and CORT in plasma, yolk or albumen were noted (Figure 3C,D,E).

4. Discussion

It is clear from this study that the equal THI value conditioned from different combinations of temperature and RH (i.e., LH75 or HL75) had identical effect on laying performance, egg quality, and serum biochemical profiles during the period of 28 days. Except for albumen CORT sampled at days 3, 7 and 28, all stress indicators including H/L ratio, CORT in plasma and both yolk and albumen were not altered by ambient temperature regimes. Thus, our results indicate that laying hens exhibits identical productive and physiological responses to ambient temperature with equal THI (i.e., 75) although it has different temperature and RH (i.e., LH75 vs. HL75). Furthermore, our study confirms the reliable THI chart as the thermal (heat) index developed for livestock and laying hens [8,18] as laying hens theoretically will

 doi:10.20944/preprints202011.0251.v1

receive same intensity of stress under the same THI value/zone (calculated with different temperature and RH).

It can be argued that the lack of responses to ambient temperature might be attributed to the environmental temperature (THI = 75 from 26ºC vs. 30ºC) being the difference in 4 Celsius. Indeed, Yahav et al. [19] reported that temperature, but not RH, is the most important factor in heat stress affecting performance and physiology of laying hens. However, the earlier study reported that laying hens housed at 30ºC vs. 24ºC performed less and exhibited altered nutrient digestibility [20]. Similarly, we observed the negative effect of heat stress on laying hens when they were exposed to 32ºC vs 27ºC [21]. In addition, temperature and RH that were reached to target range (LH75 and HL75) maintained continuously throughout the experiment, which might be more stressful to hens than cyclic or intermittent regimes. Thus, the altered responses (i.e., performance, egg quality, serum parameters, and stress indicators) could have been detected if temperature, but not RH, plays an important role in affecting performance, behavior, or physiology of laying hens in this study.

High environmental temperatures impair egg production and eggshell quality that lead to huge economic loss in global egg industry [4,22,23]. It is well known that laying hens exposed to heat stress lower feed intake to minimize heat production, and change blood flow from organs to body surface area to dissipate sensible heat [3]. In addition, heat stress impairs the ovarian function by lowering ovarian weight and number of large follicles [6,21,24]. Thus, it is considered of utmost importance to monitor thermal environments (e.g., temperature and RH) within the poultry houses and the THI chart can be employed to expect ore predict heat stress placed onto laying hens.

It has been reported that heat stress increased H/L ratio due to low lymphocytes and high heterophils [25] altered by high circulating CORT concentration leading to the redistribution

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of lymphocyte between blood and lymphoid and non-lymphoid tissue [7]. Heat stress-exposed chicken triggers the hypothalamic-pituitary-adrenal axis activation that finally raises blood CORT concentrations [26]. In this sense, CORT has been used as standard indicator to assess the impact of heat stress in poultry [5,27]. The CORT is the dominant glucocorticoid present in blood and CORT concentrations in eggs were positively correlated with maternal CORT circulation [28,29]. CORT are deposited in eggs in both a chronic manner through incorporation into the yolk during the rapid yolk deposition phase of follicular development and in an acute manner via passage into the albumen at the magnum after ovulation [17,30]. In this study, except for the elevated CORT levels in albumen sampled at days 3, 7, and 28 following heat treatment, H/L ratio and CORT levels in plasma, yolk and albumen were not altered by ambient environment during the period of heat exposure. A medium correlation was found in yolk CORT and albumen CORT (r=0.499) and low correlation was found in plasma CORT and yolk CORT (r = 0.288), CORT in plasma and albumen (r = 0.207), and H/L ratio and yolk CORT (r = 0.110). Our study suggests that both temperature- and RH-conditioned environment had equal impact on, if any, stress response in laying hens.

5. Conclusion

It is concluded from this study that laying hens exposed to ambient temperature conditioned with LH75 vs. HL75 exhibited identical laying performance, egg quality and plasma biochemical profiles. As to the stress indicators, both environment regimes failed to affect blood H/L ratio and CORT levels in plasma, yolk and albumen although albumen CORT was elevated in LH75 vs. HL75 at days 3, 7, and 28. Thus, laying hens that are exposed to equal THI values will receive similar thermal stress. Our results could be helpful in establishing mitigation guidelines for ambient temperature control of laying hens.

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Author's contributions

DHK, and KWL: contribution to conception and design, interpretation of data, acquisition of data, and analysis and drafting of the manuscript. YKL, and SHK: revising the manuscript critically. All authors have read and contributed to the final manuscript.

Funding

This work was carried out with the support of "Cooperative Research Program for Agriculture Science and Technology Development (Project No. PJ013446)" Rural Development Administration, Republic of Korea.

Competing interests

The authors declare that they have no competing interests.

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Table 1. The ingredient and chemical composition of the basal diet

Ingredients	%
Corn	43.0
Wheat	5.59
Soybean meal, 45% Crude protein	5.14
Rice dehulled	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
Tryptophane-10%	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 following nutrients per kg of diet: vitamin A, 15,400 IU; vitamin D_3, 3,080 IU; 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 provide 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.

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Table 2. Effect of different ambient temperature with equal temperature-humidity index (THI = 75) on laying performance in laying hens[1]

	Thermal treatment				*P*-value
	LH75[2]		HL75		
	Mean	SD[4]	Mean	SD	
0 to 28 days					
Hen-day egg production, %	75.73	9.70	74.82	11.39	0.849
Egg weight, g/egg	66.97	3.96	65.43	2.26	0.299
Egg mass, g/day	50.76	7.26	49.00	7.97	0.612
Feed intake, g/day/bird	115.8	5.51	117.4	7.64	0.591
FCR[3], kg/kg	2.32	0.34	2.44	0.31	0.427
FCR, kg/12 eggs	1.86	0.23	1.91	0.23	0.605

[1]n = 10 replicates per treatment

[2]LH75 = temperature 26°C; relative humidity 70%; HL75 = temperature 30°C; relative humidity 30%

[3]FCR = feed conversion ratio

[4]SD = standard deviation

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Table 3. Effect of different ambient temperature with equal temperature-humidity index (THI = 75) on heterophil/lymphocyte ratio and plasma corticosterone levels (ng/ml)[1]

	Thermal treatment				P-value
	LH75[2]		HL75		
	Mean	SD[3]	Mean	SD	
Heterophil/lymphocyte ratio					
Day 0	0.316	0.067	0.329	0.144	0.821
Day 2	0.351	0.232	0.229	0.107	0.145
Day 3	0.281	0.152	0.322	0.188	0.596
Day 7	0.220	0.127	0.230	0.151	0.875
Day 8	0.155	0.094	0.163	0.109	0.864
Day 12	0.262	0.089	0.271	0.177	0.893
Day 14	0.172	0.073	0.218	0.087	0.246
Day 16	0.349	0.221	0.282	0.161	0.447
Day 20	0.298	0.269	0.240	0.100	0.531
Day 28	0.237	0.089	0.211	0.102	0.545
Corticosterone, ng/ml					
Day 0	8.48	4.857	10.67	3.164	0.246
Day 2	8.67	2.126	8.78	3.543	0.935
Day 3	11.01	4.288	9.72	3.042	0.447
Day 7	9.78	4.486	10.23	1.406	0.791
Day 8	12.34	2.312	10.54	4.497	0.303
Day 12	8.01	3.131	8.62	2.870	0.667
Day 14	11.56	2.916	10.47	4.236	0.511
Day 16	11.61	2.102	13.13	2.525	0.160
Day 20	13.25	2.677	13.50	2.898	0.848
Day 28	14.47	2.947	14.12	1.817	0.762

[1]n = 10 replicates per treatment

[2]LH75 = temperature 26°C; relative humidity 70%; HL75 = temperature 30°C; relative humidity 30%

[3]SD = standard deviation

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Table 4. Effect of different ambient temperature with equal temperature-humidity index (THI = 75) on corticosterone levels in yolk and albumen (ng/g)[1]

	Thermal treatment				P-value
	LH75[2]		HL75		
	Mean	SD[3]	Mean	SD	
Yolk					
Day 0	12.31	0.746	11.45	1.975	0.298
Day 2	13.03	2.878	13.09	2.762	0.960
Day 3	10.56	2.826	9.05	1.472	0.151
Day 7	9.64	1.786	10.56	0.756	0.152
Day 8	10.50	3.701	9.65	2.903	0.585
Day 12	10.99	2.421	9.71	1.801	0.220
Day 14	9.03	1.178	9.64	1.495	0.323
Day 16	11.84	2.201	12.40	1.608	0.524
Day 20	13.09	3.492	13.36	2.131	0.833
Day 28	16.13	3.107	16.18	1.907	0.968
Albumen					
Day 0	14.32	2.324	18.70	2.870	0.169
Day 2	14.17	4.417	15.10	4.328	0.639
Day 3	12.58	3.389	10.04	2.707	0.096
Day 7	13.71	2.636	10.00	2.136	0.003
Day 8	7.55	3.333	9.93	5.956	0.283
Day 12	9.67	2.540	9.60	3.016	0.960
Day 14	9.70	3.692	11.43	2.076	0.213
Day 16	15.17	3.044	17.94	5.672	0.189
Day 20	15.40	3.500	15.13	4.364	0.879
Day 28	28.96	4.256	25.53	3.608	0.084

[1]n = 10 replicates per treatment

[2]LH75 = temperature 26°C; relative humidity 70%; HL75 = temperature 30°C; relative humidity 30%

[3]SD = standard deviation

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FIGURES LEGENDS

Figure 1. The effect of different ambient temperature and humidity (same Temperature-Humidity Index) on plasma biochemical parameters. Laying hens were exposed to two ambient temperature conditions (26℃, 70%; 30℃, 30%). Letters not sharing common Error bars indicate standard deviation (n = 10) and mean values sharing at least one common lowercase shown above the bars are not significantly different ($P < 0.05$). LH75 = temperature 26℃; relative humidity 70%; HL75 = temperature 30℃; relative humidity 30%. (A) Total cholesterol (mg/dL). (B) Triglycerides (mg/dL). (C) High density lipoprotein (HDL) cholesterol (mg/dL). (D) Calcium (mg/dL). (E) Phosphorus (mg/dL). (F) Magnesium (mg/dL). (G) Nitric oxide (μM).

Figure 2. The effect of different ambient temperature and humidity (same Temperature-Humidity Index) on egg quality. Laying hens were exposed to two ambient temperature conditions (26℃, 70%; 30℃, 30%). Letters not sharing common Error bars indicate standard deviation (n = 10) and mean values sharing at least one common lowercase shown above the bars are not significantly different ($P < 0.05$). LH75 = temperature 26℃; relative humidity 70%; HL75 = temperature 30℃; relative humidity 30%. (A) Haugh unit. (B) Eggshell strength (kgf). (C) Eggshell thickness (mm). (D) Eggshell weight (g). (E) Specific gravity (f/cm^3). (F) Eggshell weight (%).

Figure 3. Coefficient of correlation between (A) corticosterone in yolk and corticosterone in plasma. (B) corticosterone in yolk and corticosterone in albumen, (C) corticosterone in yolk and heterophil to lymphocyte ratio, (D) heterophil to lymphocyte ratio and corticosterone in

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plasma, (E) heterophil to lymphocyte ratio and corticosterone in albumen, and (F) corticosterone in albumen and corticosterone in plasma.

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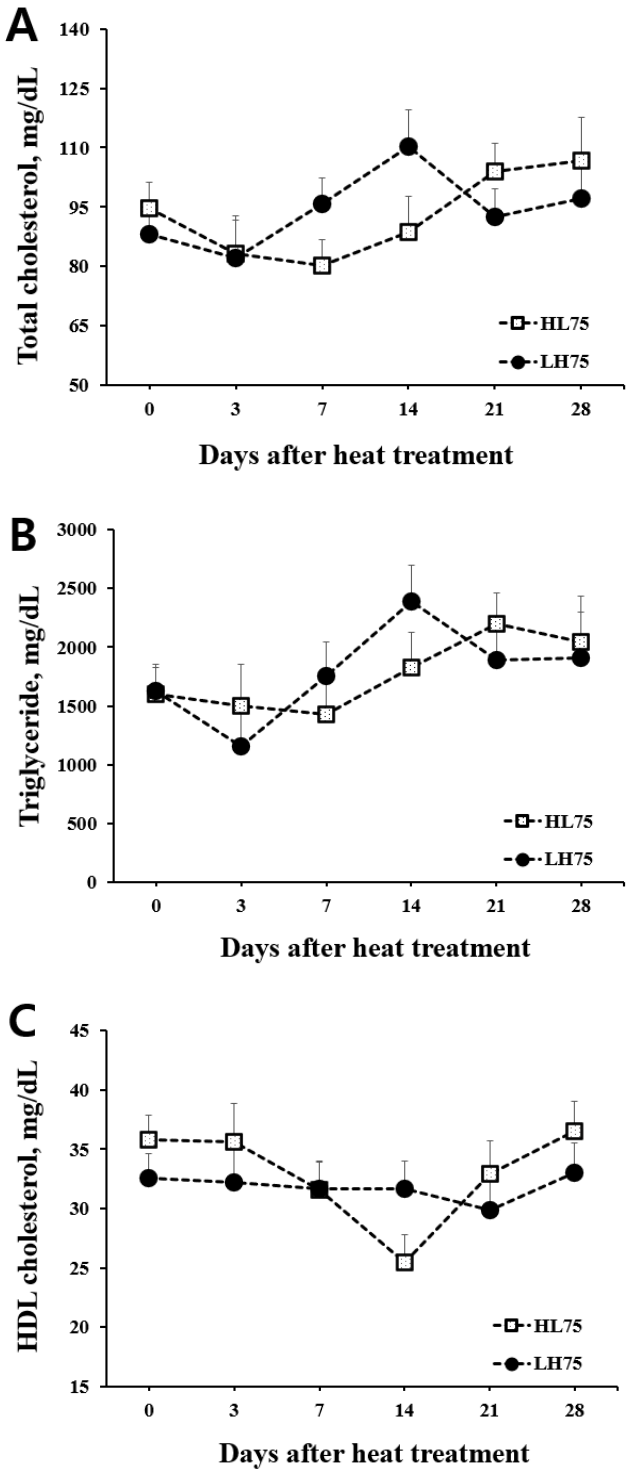
Figure 1.



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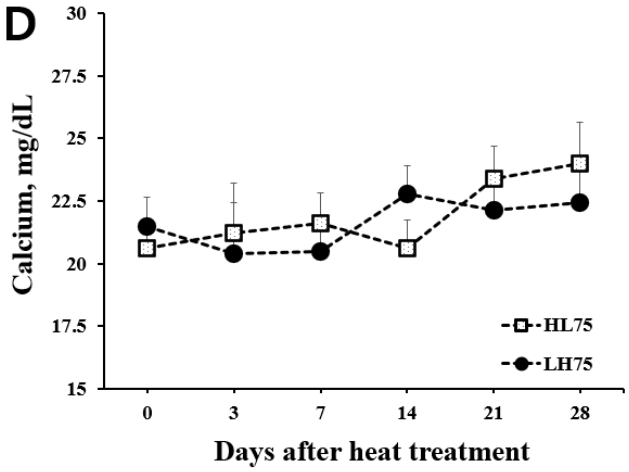

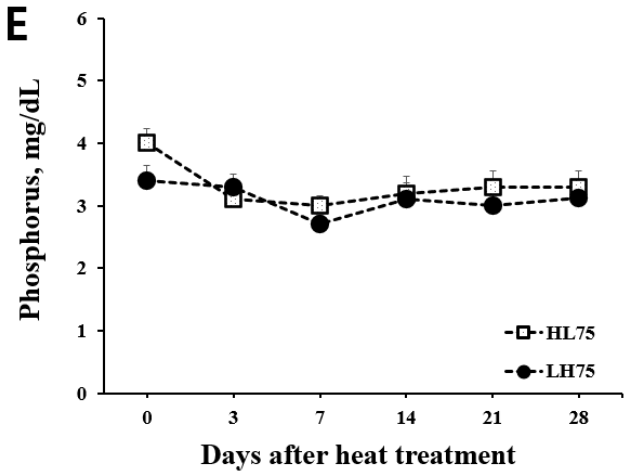

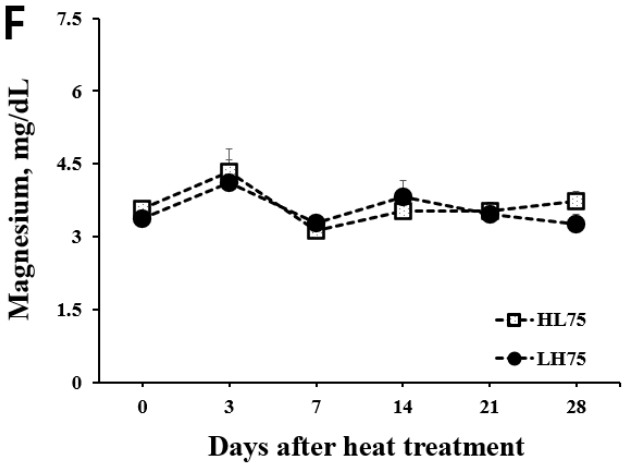

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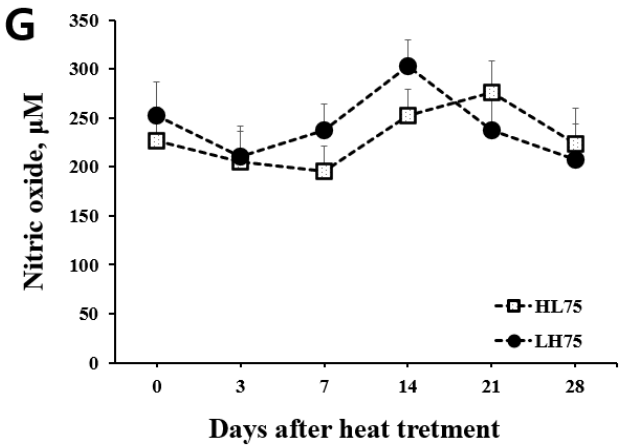

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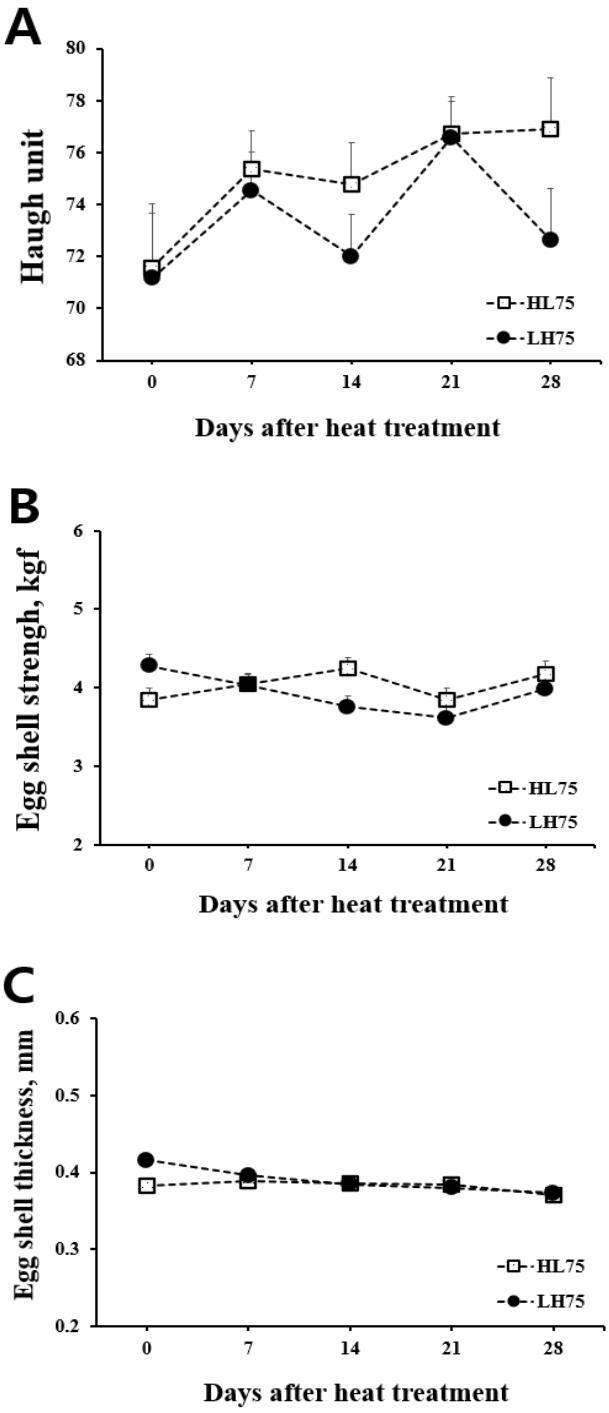

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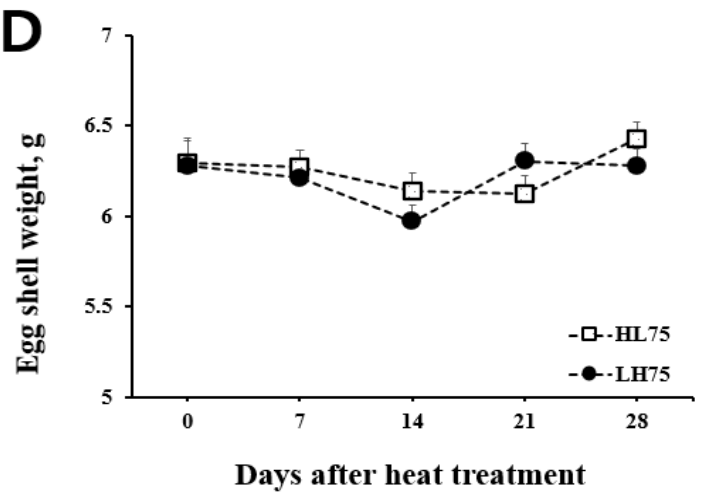

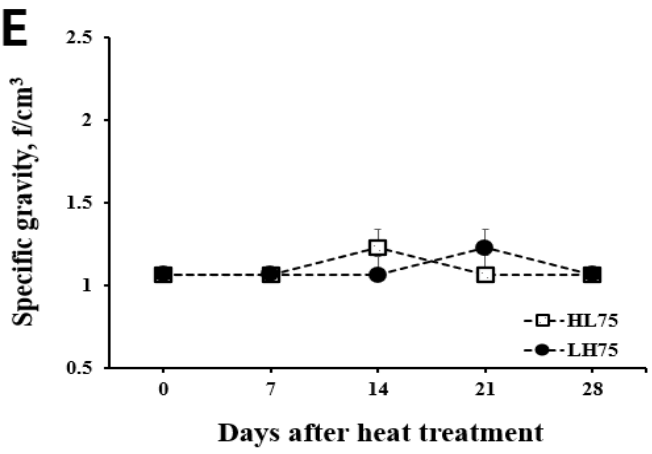

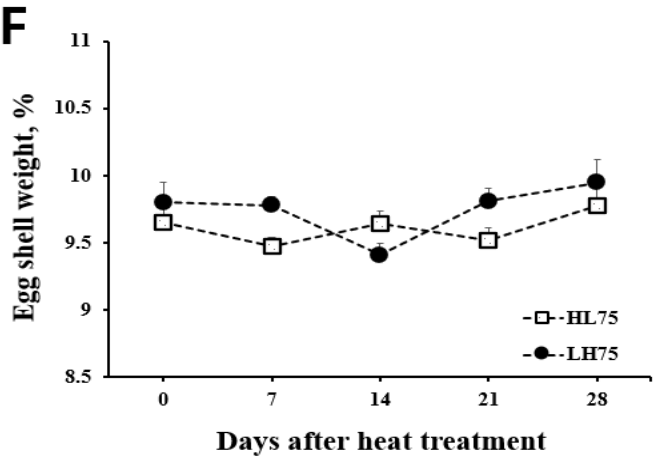

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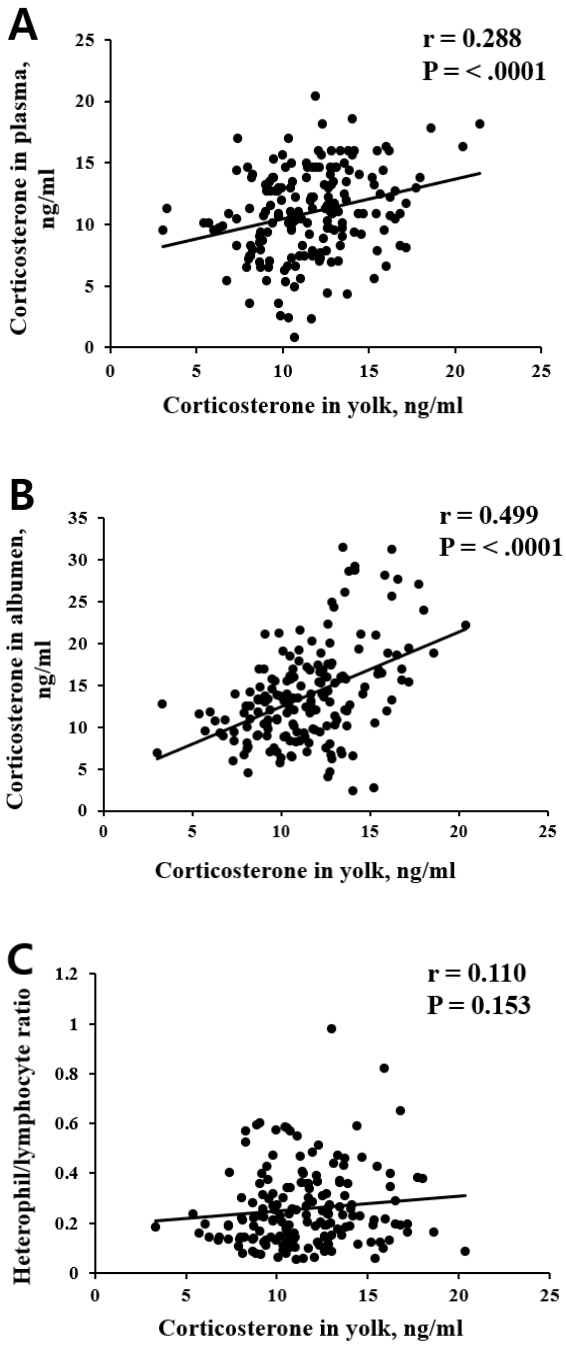
Figure 3.



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