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Impact of climate change on thermoregulation, blood attributes and production performance in laying hens

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Abstract The objective of the study was to assess the impact of climate change, expressed through the Temperature Humidity Index (THI), on physiological responses, including body temperature, blood parameters, and production indicators on laying hens, the Novogen Brown strain, from the 22nd to 34th weeks of age. The experiment was conducted in northern Algeria during the summer season in two separate buildings. The first building, which housed approximately 85,000 laying hens, was modern with a controlled environment (control group). On the other hand, the second building, housing approximately 40,000 laying hens, was exposed to natural variations in the external climate (experimental group). Additionally, the temperature fluctuated between 25.2 and 39.4°C, while humidity ranged from 54.3 to 64.5%. The overall trend of the results revealed notable variations in the parameters studied in the experimental group. Body temperature showed a significant (P<0.001) increase of +0.99°C. Blood parameters also revealed significant (P<0.001) increases; the amplitudes were +0.18 for the heterophil/lymphocyte ratio, +1.82ng/mL for corticosterone, and +5.06ng/mL for cortisol. Furthermore, production performance showed significant reductions (P<0.001) in egg weight (-4.67g) and laying rate (-6.77%) with an increase of +1.75% in the egg breakage rate (P<0.001). The results of the present study highlighted the stress that laying hens may experience due to climate change, particularly when hens are housed in buildings that do not meet the standards. These situations could compromise the sustainability of the poultry industry and threaten food security, given the importance of poultry products in the human food chain.

Keywords: climate change, laying hens, thermoregulation, blood markers, production

Introduction

Poultry are primarily bred for meat and egg production as excellent sources of complete proteins, which are more affordable compared to red meats and easily accessible, particularly in emerging countries (Erdaw and Beyene, 2022). To this end, the poultry sector continues to develop due to increased demand for low-cholesterol meat and eggs, which position them as an essential and desirable element of the human diet. With a world population of around 7.9 billion, some forecasts indicated that it could reach 9.3 billion by 2050 and 11 billion by 2100, implying a sustained demand for animal proteins (Ouchi et al., 2021). To address this, genetic selection has primarily focused on enhancing the growth and feed efficiency that

could lead to significant advancements in production. Furthermore, genetic improvement has increased the animal metabolism; intensifying thermogenesis due the presence of plumage and the lack of sweat glands (Mignon-Grasteau et al., 2015). This hypersensitivity to changes in ambient temperature is intensified when humidity fails to meet the required levels (Kim et al., 2020). Therefore, it is imperative to maintain a balance between development and concerns related to the sustainability and health of the animals. In this sense, one of the poultry industry's challenges is the perceived climate change, particularly global warming, which threatens the food security (Bayer and Barak, 2017). According to specific forecasts for 2050, equatorial, tropical, and Mediterranean regions will suffer more significant impacts from climatic upheavals, resulting in more intense and prolonged heat waves. This is also the case in Algeria, where the increase in temperature ranged from 1.5 to 2°C during the 20th century, higher than the global average of 0.74°C, as well as reduced precipitation by 10 to 20% (Yerou et al., 2021).

Furthermore, Nawab et al. (2018) pointed out that in most cases, conditions in commercial poultry farms expose the birds to many stress factors, including thermal stress. Inappropriate ambiance control in poultry houses intensifies the impact of heat stress on birds. This is the case in most countries on the southern shore of the Mediterranean, including Algeria, where breeding is carried out in dilapidated structures that are highly heterogeneous and small in size. Poultry buildings are, with rare exceptions, of the clear type, with static ventilation and poor insulation, and most poultry farmers do not control the environmental parameters. These specific conditions can lead to situations where the body's demands surpass its natural ability to regulate the body temperature (Campderrich et al., 2019). This can negatively impact on the bird's well-being (He et al., 2018) by increasing the morbidity and mortality rates (Nidamanuri et al., 2017), resulting in significant economic losses. Given all these considerations, this study aimed to examine the repercussions of climate change, expressed by the THI (Temperature Humidity Index), on body temperature, blood markers, and production performance in laying hens bred in the Algerian context.

Materials and methods

Ethical statement

The experiment was approved by the Faculty of Nature and Life Sciences and Earth Sciences, Djilali Bounaama University, Khemis Miliana. The experiment lasted 84 days, from June 1st to August 24th, 2023.

Experimental design

The hens, Novogen Brown strain at 18 weeks of age, were housed in two separate buildings. A standard building providing controlled environment, was used to house 85,000 hens, distributed into 5 per cage (control group, group C). The second building that was equipped only with ventilation and pad cooling systems housed a population of 40,000 hens, equally distributed at 5 per cage (the experimental group, group E). The hens received a standard diet for each production phase (Table 1). Measurements were taken every 15 days for 12 weeks, between the 22nd and 34th weeks of age. Parameters measured included the production performance (laying rate, egg weight, breakage rate, and mortality rate), body temperature, and blood parameters (heterophil/lymphocyte ratio: H/L, corticosterone, and cortisol).

Table 1. Nutritional composition of the diet during the experiment

Composition	Amount
Metabolizable energy (kcal/kg)	2700 - 2800
Crude proteins (%)	17 - 18
Fat (%)	4 - 7
Crude fiber (%)	7
Lysine (%)	0.60
Methionine (%)	0.27
Tryptophan (%)	0.14
Calcium (%)	3.5
Phosphorus (%)	0.31
Sodium (%)	0.13

Ambient parameters

Ambient temperature and relative humidity were measured using recording thermo-hygrometers placed at different locations in the buildings. The devices recorded data every half hour. During the experiment, the control group was maintained under an average temperature of 19.70±2.18°C and relative humidity of 65.93±2.98%. Moreover, the experimental group was raised under a temperature of 31.69±2.54°C and a relative humidity of 57.22±2.39%. The THI index was calculated using temperature (T, °C) and humidity (H, %) measurements (Kendall and Webster, 2009) as:

$$THI = (1.8T + 32) - [(0.55 - (0.0055 \times H)) \times (1.8T - 26)]$$

The THI was 64.03 for the control group and 79.61 for the experimental group.

Body temperature

At the start of the experiment, ten hens per group were selected and identified to practice this measurement. An electronic thermometer was inserted into the cloaca to a depth of 2 cm; this was thoroughly cleaned with water and antiseptic between before each temperature measurement to avoid contamination.

Blood parameters

Ten laying hens were selected per building. Blood was collected from the wing vein in previously identified EDTA and heparinized tubes 12 h after fast, immediately centrifuged at 3000 rpm for 15 minutes, plasma was collected with micropipettes fitted with disposable tips and kept in Eppendorf-type tubes. The H/L ratio was

determined after measuring the heterophils and lymphocytes by a colorimetric method (Alan, 2006) based on counting the cells using an ADVIA 560-type automated system at a wavelength of 546 nm. Hormonal parameters were measured by the enzyme immunoassay method (Eastman et al., 1975) using an AIA360-type automatic analyzer. Corticosterone was determined with the kit (DRG Corticosterone ELISA: EIA-4164), and cortisol with the kit (DRG Cortisol ELISA: EIA-1887). The sensitivity of the kit was 0.5 ng/mL for corticosterone and 2.5 ng/mL for cortisol, respectively.

Production performance

The laying rate was calculated by dividing the number of eggs laid daily by the number of hens present. Egg weight was measured using a random sample of 90 eggs (Pan Scale manufactured by KERN, with a precision of 0.01g). The breakage rate was calculated by dividing the number of abnormal eggs (cracked and/or broken) by total eggs. Mortality rate was calculated as the ratio of dead hens to the total number of hens present.

Statistical analysis

The data were expressed as the means ± standard deviations. Calculations were carried out using Microsoft Excel software, version 2007. Before the statistical analysis, the intra-assay and inter-assay coefficients of variation (CV, %) were calculated for each parameter. Furthermore, the data distribution curve was plotted for each parameter studied using the same software. This also made it possible to calculate the coefficient of determination of the linear regression (R^2), the results of which are presented in Table 2.

Table 2. Coefficient of determination of the linear regression (R^2) for measured parameters in laying hens

Parameters	Group C	Group E
Body temperature	0.91	0.950
Heterophil/lymphocyte ratio	0.876	0.969
Corticosterone	0.890	0.953
Cortisol	0.844	0.935
Egg weight	0.903	0.897
Laying rate	0.883	0.876
Breakage rate	0.982	0.934
Mortality rate	0.791	0.962

Group C: Birds housed in controlled environment
Group E: Birds kept natural variations in the external climate

After ensuring that the parameters followed the normal distribution law, as evidenced by the R^2 values exceeding 0.80, the statistical difference between the two groups was determined using the Student's t-test. The level of significance was set at $P \leq 0.05$.

Results

Body temperature

The effect of the Temperature Humidity Index (THI) on the body temperature of laying hens is shown in Table 3. Body temperature was significantly higher in the hens of the experimental group compared to the control group for the different samples. Furthermore, the highest increase approached +1°C.

Table 3. Effect of THI on body temperature (°C) in laying hens at different weeks of age

Week of age	Group C	Group E	P-value
W22	41.23±0.27	41.87±0.32	0.00015
W24	41.00±0.34	41.90±0.47	0.00014
W26	41.34±0.37	41.84±0.38	0.0093
W28	41.03±0.51	41.78±0.46	0.0030
W30	40.92±0.57	41.91±0.35	0.00020
W32	41.25±0.25	41.77±0.36	0.0015
W34	41.37±0.26	41.83±0.27	0.0012

Group C: Birds housed in controlled environment
Group E: Birds kept natural variations in the external climate

Blood parameters

Heterophil/lymphocyte ratio

Heterophil/lymphocyte ratios revealed increasing trends in the experimental group compared to the control group (Table 4). Significant increases were noted, particularly at the end of the experiment, with a maximum difference of +0.18 in absolute value, representing +28.57% proportionally.

Table 4. Effect of THI on heterophil/lymphocyte ratio in laying hens at different weeks of age

Week of age	Group C	Group E	P-value
W22	0.33±0.04	0.47±0.03	>0.05
W24	0.34±0.07	0.48±0.09	0.0028
W26	0.31±0.01	0.34±0.01	0.00010
W28	0.35±0.02	0.53±0.02	>0.05
W30	0.33±0.03	0.57±0.04[a]	>0.05
W32	0.41±0.05	0.62±0.08	>0.05
W34	0.45±0.09	0.63±0.06	0.00012

Group C: Birds housed in controlled environment
Group E: Birds kept natural variations in the external climate

Glucocorticoids

The impact of THI on corticosterone and cortisol levels is reported in Table 5. Regarding corticosterone, notable and significant increases were observed in the laying hens of the experimental group compared to the control group. Moreover, the largest increase observed was +1.82 ng/mL, representing a proportional augmentation of +16.74%. Cortisol showed a trend similar to that of corticosterone, with significant increases in the experimental group. The most notable difference reached +5.06 ng/mL, representing a proportional increase of +15.90%.

Production performance

The influence of THI on egg weight is reported in Table 6. Laying hens in the experimental group produced eggs with significantly lower average weight for most of the samples. The maximum recorded difference was significant, with an absolute reduction of -4.67 g and a proportional decrease of -8.34%.

Table 5. Effect of THI on corticosterone and cortisol levels (ng/mL) in laying hens at different weeks of age

Week of age	Corticosterone			Cortisol		
	Group C	Group E	P-value	Group C	Group E	P-value
W22	9.53±0.16	10.03±0.36	0.00088	25.55±0.62	28.07±1.47	0.00010
W24	9.37±0.39	10.57±0.75	0.00030	26.75±2.33	31.81±2.49	0.00018
W26	9.40±0.41	11.22±1.08	0.00010	27.19±2.54	31.38±1.85	0.00053
W28	9.05±0.99	10.87±1.06	0.00095	25.90±2.69	31.61±1.91	>0.05
W30	9.10±0.44	11.61±0.81	>0.05	26.39±1.58	32.04±2.55	>0.05
W32	10.77±0.96	12.91±1.21	0.00037	29.14±2.17	32.07±2.43	0.011
W34	11.43±0.99	13.36±0.92	0.00028	29.40±2.28	33.67±2.86	0.0017

Group C: Birds housed in controlled environment
Group E: Birds kept natural variations in the external climate

Table 6. Effect of THI on egg weight (g) in laying hens at different weeks of age

Week of age	Group C	Group E	P-value
W22	61.82±3.01	57.21±2.44	0.0014
W24	60.66±2.21	55.99±2.38	0.00025
W26	60.95±1.76	57.72±1.67	0.00054
W28	61.39±1.70	58.01±1.50	0.00018
W30	61.51±1.59	57.14±1.87	>0.05
W32	61.60±2.12	58.58±1.80	0.0029
W34	61.20±2.83	58.50±1.79	0.020

Group C: Birds housed in controlled environment
Group E: Birds kept natural variations in the external climate

The impact of THI on laying and breakage rates is presented in Table 7. Hens in the experimental group produced fewer eggs compared to the control group. The largest difference was significant, with a reduction of -6.77% in absolute value and -8.13% proportionally. In contrast, the experimental group producing more cracked and/or broken eggs. In terms of absolute value, the largest increase was significant, with a difference of +1.75%, representing a proportional increase of +31.81%. Mortality rate was non-significantly higher in the experimental group (Table 8).

Table 7. Effect of THI on laying rate and breakage rate (%) in laying hens at different weeks of age

Week of age	Laying rate			Breakage rate		
	Group C	Group E	P-value	Group C	Group E	P-value
W22	89.26±2.29	84.09±2.47	0.00013	3.46±0.23	5.56±0.33	>0.05
W24	87.74±2.30	83.52±2.03	0.00040	3.63±0.39	6.06±0.63	>0.05
W26	89.45±1.97	83.95±2.30	>0.05	3.48±0.27	5.15±0.55	>0.05
W28	90.06±3.71	84.30±2.95	0.0012	3.92±0.36	5.49±0.28	>0.05
W30	88.99±4.00	82.80±3.37	0.0015	3.71±0.61	5.66±0.62	>0.05
W32	90.09±4.04	84.93±2.14	0.0022	3.76±0.69	5.51±0.92	0.00014
W34	90.01±3.71	83.24±2.99	0.00028	4.00±0.53	5.53±0.84	0.00013

Group C: Birds housed in controlled environment
Group E: Birds kept natural variations in the external climate

Table 8. Effect of THI on mortality rate (%) in laying hens at different weeks of age

Week of age	Group C	Group E	P-value
W22	1.06±0.07	1.40±0.09	>0.05
W24	1.20±0.08	1.60±0.09	>0.05
W26	1.32±0.09	2.13±0.10	>0.05
W28	1.18±0.10	2.46±0.10	>0.05
W30	1.05±0.06	2.40±0.11	>0.05
W32	1.32±0.08	2.42±0.09	>0.05
W34	1.21±0.07	2.52±0.09	>0.05

Group C: Birds housed in controlled environment
Group E: Birds kept natural variations in the external climate

Discussion

The study aimed to highlight the repercussions of climate change, expressed through the THI, on the physiological responses Novogen Brown hens. In contrast to the experimental group, the control group was maintained under optimal conditions, as indicated by temperature and relative humidity readings. The breeding guide for this strain recommends ambient temperatures of 17 to 19°C and relative humidity levels of 60 to 70% from eight weeks of age (Novogen Brown, 2015). Additionally, the THI index was 64 for the control group and rose to nearly 80 for the experimental group, indicating that the experimental group experienced heat stress. Zulovich and De Shazer (1990) developed THI thresholds for laying hens based on production levels and physiological responses. These thresholds categorize the stress experienced by hens into four levels: comfort zone (<

70), alert zone (70-75), danger zone (76-81), and emergency zone (> 81). Kang et al. (2020) indicated that increased THI negatively impacts laying hens by causing hyperventilation, which leads to higher mortality rates. The same authors concluded that THI is a reliable tool for assessing the effects of heat stress on laying hens.

Impact of THI on body temperature

The study indicated that increasing ambient temperature, which increased THI, led to significant increases in body temperature. These results agree with the study by Al-Dawood and Al-Tamimi (2022), who reported that thermal stress, whether constant or cyclic, caused an increase in body temperature in laying hens. This general trend was also observed in broiler chickens (Bueno et al., 2017) and Japanese quails (Attia et al., 2021). Taylor et al. (2014) and Chang et al. (2018) linked this reaction to the physiological status of the hens. Hens are homeotherms whose thermoregulatory system balances the processes of thermogenesis and thermolysis to adjust their body temperature. This specificity allows hens to maintain their body temperature constantly, regardless of the ambient temperature. Furthermore, Pawar et al. (2016) indicated that ambient temperatures between 19 and 22°C allow normal physiological functioning in laying hens. However, beyond these limits, the thermoregulatory system is activated to stabilize the body temperature (Chang et al., 2018). Under more severe conditions, this system may be unable to restore homeothermy, causing serious physiological consequences, as noted by Saeed et al. (2019), who observed that a 4°C increase in body temperature could be fatal for laying hens.

Impact of THI on blood parameters

High THI significantly elevated the H/L ratio. This trend is consistent with the study by Melesse et al. (2011). Strong (2014) also observed this trend in laying hens under heat stress compared to those with access to cooled perches. The results also showed that corticosterone and cortisol levels increased significantly under high THI. He et al. (2018) and Mishra et al. (2019) noted that laying hens raised under heat stress showed significantly increased serum corticosterone. Moreover, exploring cortisol as an indicator of stress has received less attention in birds, with corticosterone being the focus of most research. Recent studies have shown that cortisol levels increased significantly under heat stress conditions, whether chronic or acute, in broilers (Mirsaiidi Farahani and Hosseinian, 2022), Japanese quails (Nassar et al., 2023), and ducks (Oluwagbenga et al., 2022). Such responses could be due to heat stress in the experimental group, which triggered chain reactions to create adaptive pathways to heat stress. Indeed, glucocorticoids are essential in implementing stress responses and controlling the variability of neuroendocrine and immune responses (Nazar et al., 2012). Glucocorticoid secretion increases sharply following activation of the sympathetic and hypothalamic-pituitary-adrenal axis (Kolesnik and Derkho, 2018). Glucocorticoids also act on immune cells (Nidamanuri et al., 2017). Increases in heterophils reflect the severity of the primary response to stressors and decreases in lymphocytes, thereby increasing the H/L ratio (Hosseini-Vashan et al., 2016), resulting from rapid apoptosis of lymphocytes induced by these hormones (Campderrich et al., 2019). In addition, glucocorticoids play a fundamental role in the formation of a physiological pathway of adaptation; they are associated with their ability to control catabolic processes of metabolism and determine the level of energy released, as well as the adequacy of their use for the restoration of homeostasis (Kolesnik and Derkho, 2020). Furthermore, immune cells have receptors for these hormones (El-Lethey et al., 2003), and suppressing the immune response decreases the body's resistance to microbes (Dohms and Metz, 1991).

Impact of THI on production performance

The increase in glucocorticoids in the blood negatively impacted on production performance. Significant reductions in laying rate and egg weight and an increase in breakage rate were noted. This trend was also observed by Allahverdi et al. (2013) after exposing laying hens to heat stress for 13 weeks. Furthermore, heat stress significantly decreased egg production (Barrett et al., 2019) and shell quality (Oguntunji and Alabi, 2010). The decreased production performance could result from the reorientation of energy expenditure towards maintaining thermal neutrality (Zaboli et al., 2019) following the activation of the hypothalamic-pituitary-adrenal axis. These deleterious conditions would increase the respiratory rate (Renaudeau et al., 2011), which in turn promotes an acid-base and electrolyte imbalance (Borges et al., 2004; Imik et al., 2013). Increased panting leads to decreased blood carbon dioxide levels and increased blood pH (alkalosis), limiting the availability of bicarbonate for eggshell mineralization (Sahin et al., 2002). This makes the eggs more brittle and increases the breakage rate. Moreover, the mortality rate revealed an increasing trend but remained statistically insignificant. These findings agree with studies by Kim and Lee (2023) and Kim et al. (2024), which indicated that chronic heat stress affected the metabolic and endocrine responses but did not necessarily lead to significant increases in mortality rates. Additionally, Kang et al. (2020) indicated that gradual increases in THI do not significantly increase the mortality rates. The same authors suggested that the level of temperature increase is a determining factor in the physiological responses of laying hens. The data from this study support the results of previous research on the impact of heat stress and THI on the immune, endocrine, and physiological responses of laying hens.

Conclusions

Extreme climate change, expressed through THI, caused physiological disturbances that ultimately affected production indicators in laying hens raised in non-controlled conditions. These findings also highlighted the importance of controlling environmental parameters in the Mediterranean context to ensure optimal production. Ultimately, neglecting environmental conditions could seriously compromise the sustainability of poultry production, endangering the supply of eggs and threatening the food security. To limit these detrimental effects, further research should focus on developing cost-effective and sustainable strategies, such as feed supplementation or genetic selection for heat tolerance, to improve the resilience of laying hens facing thermal stress.

Conflict of interests

There was no conflict of interest.

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