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## Research Article Evaluation of Egg Quality Indices, Fertility, Hatchability % and Some Biochemical Parameters under the Effect of Some Environmental Stressors in Laying Hen

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### Abstract

**Objective:** The aim of the present study was to investigate the effect of some environmental stressors (temperature and humidity) on egg quality indices, fertility and hatchability % and some biochemical data in laying hen. **Materials and Methods:** One hundred and eighty adult laying hens at 20 weeks of age were selected and used in this current experiment. The birds were randomly divided into three groups of 60 each and each group was further divided into five replicates of 12 birds. Three stages of thermal comfort values were established such as: Normal  $\leq$ 74, alert from 75-78, danger values are those from 79-83. **Results:** Control group (which housed at normal THI  $\leq$ 74) achieved the highest significant egg quality traits, egg efficiency indices, comparing to thermally stressed groups. Exposure of birds to high THI (T2) causes a significantly (p<0.01) decrease in the fertility and hatchability %. It was noticed that thermal stress could significantly (p<0.01) affect serum levels of T3 to reach the peak value in the stressed group. On the reverse directions, the serum level of T4 achieved the highest significant level in the control group and the lowest value was achieved in thermally stressed groups. On the same way, the level of plasma proteins or lipid profile showed a highly significant (p<0.001) increase in control group than groups exposed to thermal stress. Corticosterone level achieved the high level in thermally stressed groups. In addition, TLC showed the highest significant level in the control group. **Conclusion:** Laying hen exposed to high environmental conditions in order to maintain the production and consequently the economic income.

Key words: Egg quality, fertility, hatchability, environmental stressors, laying hen

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

#### INTRODUCTION

The poultry industry is one of the main agricultural industries in the world as it plays an important role in the economic income and it is an important source in human nutrition and employment. Poultry is the largest livestock group and consisting of approximately 252.3 million birds including chickens, ducks, geese, pigeons etc<sup>1</sup>. Egg quality indices are very important parameter from the productive point of view, there are numerous factors affecting this quality (external or internal) include nutrition, age, stress and disease. But the most important factor which should be managed for the bird's production, welfare, good quality and to decrease the losses in egg production (10% of total egg production) is the heat stress<sup>2</sup>.

At the bird's productive life, heat stress is a major management problem as the laying hen will not be able to continue their normal performance when there is an increase in body temperature. Plus, the negative effect of heat stress on egg efficiency, egg weight and egg guality traits<sup>3-5</sup>. Little attention has received toward the responses of poultry to high relative humidity and it is rarely included as an experimental variable or even measured for information purposes comparing to high ambient temperature which received a great deal of knowledge especially when accompanied by high humidity, impose severe stress on birds and lead to decrease the laying performance<sup>6</sup>. All data about humidity is critical especially in regions producing poultry as high temperatures can often be accompanied by a range of RH, which can markedly affect the degree of heat stress experienced by the birds7. It has been reported that a high temperature accompanied by high humidity is more detrimental to layer performance than a high temperature with low humidity<sup>8</sup>.

However, to monitor heat stress-related losses temperature humidity index (THI) was adapted for the first time, using wind speed as a variable and it is called the temperature-humidity-velocity index (THVI)<sup>9</sup>. A (THI) is a single value representing the combination of air temperature and humidity associated with the level of thermal stress. Utilizing both humidity and temperature is important compared with utilizing temperature alone to evaluate heat stress. Therefore, this research aimed to evaluate the effect of some thermal indices (relative humidity and heat stress) on egg quality (internal and external), fertility, hatchability and some biochemical parameters in laying hen.

#### **MATERIALS AND METHODS**

**Experimental Birds and management:** This study was reviewed and approved by the Animal Care and Welfare

Committee of Mansoura University, Egypt. It was conducted at the Experimental Unit related to Animal Husbandry and Wealth Development Department of Faculty of Veterinary Medicine, Mansoura University. This experimental unit was provided with all equipments necessary to meet the requirements for each experimental group.

One hundred and eighty adult laying hens at 20 weeks of age were selected and used in this current experiment. The birds were randomly divided into three groups of 60 each and each group was further randomly divided into 5 replicates of 12 birds with 1 male: 2 females mating ratio. The diet was formulated according to NRC<sup>10</sup> recommendations. The lighting schedule was 16 h light: 8 h dark for the whole experimental period. Three stages of thermal comfort values were established such as normal  $\leq$ 74, alert from 75-78, danger values are those from 79-83. These thermal comfort values were calculated<sup>11</sup> and then modified<sup>9</sup> using the following equation:

 $THI = (0.85 \times DBT + 0.15 \times WBT)$ 

Where:

THI = Temperature, relative humidity index DBT = Dry bulb temperature (°C) WBT = Wet bulb temperature (°C)

#### Data measurements Production parameters

**External egg quality:** Fifteen eggs per replicate were selected at random weekly for egg quality analysis. Eggs were collected daily, weighed individually to the nearest mg and recorded daily for 18 weeks. Total egg number counted and recorded daily. Egg mass/hen/week was calculated using the following formula<sup>2</sup>:

Egg Mass/hen = Egg weight×Egg number

**Internal egg quality:** Eggs were individually weighed, then broken and the inner contents were placed on a levelled glass surface to determine yolk and albumin grade. Egg shell, yolk and albumin were separated and weighed on a fresh matter basis; HU<sup>12</sup> was determined according to the following equation:

#### $HU = 100 \log (H+7.57-1.7 W 0.37)$

Yolk height was measured in mm using a tripod  $\mu$ m, yolk width was measured in mm using a vernier caliper and yolk index was calculated from the equation:

Yolk index =  $\frac{\text{Yolk height}}{\text{Yolk diameter}^{13}}$ 

Eggshell thickness was measured by a means of micrometer as an average of 3 points (top, medial and base).

**Fertility and hatchability indices:** The eggs were collected daily and stored at room temperature for up to 4 days before they were placed in the incubator. Eggs are incubated automatically, with controlled humidity (60%) and temperature (37.8°C) parameters<sup>14</sup>. On the 15th day, an accurate assessment of the developmental stage of the embryo by candling was done, numbers of eggs containing dead embryos, those containing no embryo were counted and the percent of fertility was calculated. Hatching process usually started on 19th day finished by the end of 21st day, the chicks were removed and counted. Fertility and hatchability percentages were calculated<sup>15</sup> as the following:



Hatchability (%) =  $\frac{\text{Number of hatched chicks}}{\text{Number of fertile eggs}} \times 100$ 

#### **Biochemical data**

**Corticosterone concentration and Thyroid hormones:** Serum levels of thyroid hormones (T3 and T4) were determined by ELIZA methods using commercial kits supplied by Pishtaz Teb Diagnostic, Germany<sup>16</sup>. Corticosterone was assayed by a solid phase radioimmunoassay (RIA) procedure using corticosterone commercial kit<sup>17</sup>.

**Total protein, albumin and globulin:** Total protein (TPs), albumin and globulin were measured in the serum by UV-calorimetric spectrophotometric method using commercial kits (Vitro Scient, Egypt)<sup>18</sup>.

**Total leucocytic count:** By using a small drop of blood and two slides (plain and spreader), a thin blood film was prepared. The leucocytic count blood film was performed

using the improved hemocytometer and Natt and Herrick solution as a special diluent for chicken's blood<sup>19</sup>.

**Statistical analysis:** Data were analyzed using general linear model (GLM) procedure of the Statistical Analysis System package<sup>20</sup>. Preliminary test was applied to the percentage data before comparison and analysis and found that data were homogeneous and did not need a transformation to the corresponding arcsine angle. Preliminary test was also applied and found a non-significant effect of replicate. All data were expressed as the least square mean (LSM)±S.E. p<0.05 was considered to be statistically significant.

#### RESULTS

Effect of some environmental stressors on egg quality indices: The present study showed that control group (which housed at normal THI  $\leq$ 74) achieved the highest significant egg quality traits (egg number, weight and mass, respectively) comparing to thermally stressed groups (Table 1).

Regarding egg efficiency indices, our results revealed that there was a highly significant difference (p<0.01) between experimental groups due to different THI levels. Control group achieved the highest significant parameters of egg efficiency followed by those kept at medium temperature compared to the other heat stressed group (Table 2).

Birds exposed to high THI (T2) causes a significantly (p<0.01) decrease in the fertility (73.81%) comparing to (85.44%) the control group and the medium group (80.10%), respectively (Table 3). On the same respect, hatchability percentages in the control group (80.2%) and T1 group 79.01%) were significantly (p<0.001) higher than those reported in the T2 group (65.08%) (Table 3).

In this study, the serum levels of T3 and T4 were determined in different three groups of thermal stress. It was

| Table 1: Effects of temperature I | humidity index l | level on the egg traits |
|-----------------------------------|------------------|-------------------------|
|                                   |                  |                         |

| Treatments     | Egg weight                | Egg number              | Egg mass                  |
|----------------|---------------------------|-------------------------|---------------------------|
| Control        | 61.80±0.40ª               | 85.09±0.69ª             | 5258.55±0.88ª             |
| T1             | 59.11b± 0.57 <sup>ь</sup> | 73.45±0.66 <sup>b</sup> | 4341.62±0.85 <sup>b</sup> |
| T2             | 57.70±0.55°               | 70.32±0.64°             | 4057.46±0.77°             |
| p-value        | <0.001                    | <0.001                  | <0.001                    |
| *Moons of diff | oront lovals within the   | samo column baving      | different superscripts    |

\*Means of different levels within the same column having different superscripts are significantly different (p<0.05)

Table 2: Effects of temperature humidity index level on egg efficiency indices

| Treatments | HU (mm)                 | YI (mm)                 | Shell thickness          | Yolk height            | Yolk diameter           |
|------------|-------------------------|-------------------------|--------------------------|------------------------|-------------------------|
| Control    | 83.66±0.44ª             | 43.57±0.66ª             | 37.20± 0.50 <sup>a</sup> | 1.56ª±0.08ª            | 3.58°±0.11°             |
| T1         | 72.31±0.36 <sup>b</sup> | 38.22±0.57 <sup>b</sup> | 35.40±0.57 <sup>b</sup>  | 1.49±0.06 <sup>b</sup> | 2.55±0.15 <sup>b</sup>  |
| T2         | 65.07±0.33°             | 36.15±0.59°             | 30.33±0.49°              | $1.41\pm0.05^{\rm bc}$ | 2.53±0.22 <sup>bc</sup> |
| p-value    | <0.001                  | <0.001                  | <0.001                   | <0.05                  | <0.05                   |

\*Means of different levels within the same column having different superscripts are significantly different (p<0.05)

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Fig. 1(a-f): Serum levels of metabolic biochemical parameters (thyroid hormones, total protein, albumin and lipid profile) in different levels of thermal stress.

Table 3: Effects of temperature humidity index level on fertility and hatchability (%)

| Treatments       | Control     | T1                      | T2          | p-value |
|------------------|-------------|-------------------------|-------------|---------|
| Fertility (%)    | 85.44±0.10ª | 80.10±0.13 <sup>b</sup> | 73.81±0.12° | <0.01   |
| Hatchability (%) | 80.10±0.09ª | 79.01±0.11⁵             | 65.08±0.14° | < 0.001 |

\*Means of different levels within the same column having different superscripts are significantly different (p<0.05)

noticed that thermal stress could be significantly (p<0.01) affect serum levels of T3, corticosterone and TLC to reach the peak value in stressed group (T2) and minimal value in control group. On the other hand, serum level of T4 achieved the

highest significant level in the control group and the lowest value was achieved in T2. On the same way, the level of plasma proteins or lipid profile showed a highly significant (p<0.001) difference between groups as it increased in the control group than the stressed groups (Fig. 1).

#### DISCUSSION

The aim of this study was to investigate the effect of different levels of environmental stressors on egg quality

indices, fertility, hatchability % and some biochemical parameters. Egg quality traits were affected by high thermal stress. These results were in agreements with those reported by Okpara *et al.*<sup>21</sup> and Talukder *et al.*<sup>22</sup> who reported that if relative humidity is allowed to increase normal limits for laying hens, then laying performance will be negatively affected. Also, a daily decrease in the egg production under heat stress was recorded by Karaman *et al.*<sup>23</sup>.

Moreover, the reproductive activities and egg quality in Japanese quails and laying hen will be reduced under the effect of high ambient temperatures<sup>24-26</sup>. This reduction in reproductive performance associated with heat stress is a wellknown phenomenon in domestic birds<sup>27</sup>. As during high thermal temperature; to decrease the body temperature and body heat production, birds limit their daytime activity and decrease feed consumption or even stop eating which reflected on the bird performance and profitability due to the direct debilitating effect of high ambient temperature on ovarian function in the birds<sup>4</sup>. A possible mechanism for the reduction of ovarian function might be the reduction in blood flow to the ovary; a differential ovarian blood flow pattern was found in hens exposed to high ambient temperatures, Mashaly et al.<sup>3</sup>. Moreover, Arad et al.<sup>28</sup> stated that higher temperature reduces the productive performance of layer hens.

On the contrary, Yahav *et al.*<sup>29</sup> stated that egg production was not significantly affected by increased ambient temperature. However, in most cases it was maintained or even increased; it declined in older hens exposed to 60% RH.

Moreover, Sloan and Harms<sup>30</sup> reported that there is no effect of low temperature on egg shell thickness.

**Egg efficiency indices:** Egg efficiency indices (Haugh unit, yolk index and shell thickness) will be negatively affected by high thermal stress. This may be attributed to the effect of high temperature and humidity which leading to weakness of egg yolk and liquefaction of the albumin and thereby lower values of yolk index and Haugh unit. These results were in accordance with Fouda *et al.*<sup>2</sup>, Ghrib<sup>31</sup> and Altan and Oguz<sup>32</sup> who concluded that high temperature at 33-36°C had a significant reduction in shell thickness and produced eggs with less shell thickness and inferior interior quality (Haugh Unit and yolk color scores) as compared to the control ones. In contrast, Gultekin *et al.*<sup>33</sup> showed that heat stress had no effects on Haugh unit and yolk index in Japanese quails.

**Reproductive traits (fertility and hatchability %):** The effect of thermal stress on fertility and hatchability %, when compared to control group, was detrimental in this study.

These may be in accordance with a series of experiments in Japanese quail, Ozcelik *et al.*<sup>15</sup>, Yerturk *et al.*<sup>34</sup> and Zaki<sup>35</sup>, who reported that the control group showed significant higher (p<0.05) fertility and hatchability % compared to heat stressed groups which could be explained as a result of high temperature and decreased of egg production. On the other hand, Muiruri and Harrison<sup>36</sup> and Avci *et al.*<sup>37</sup> noted non-significant effect of heat stress on hatchability of fertile eggs of Japanese quails.

**Biochemical parameters:** Control group showed the highly significant (p<0.01) lower tri-iodothyronine (T3) concentration, followed by T1 group, which is also followed by T2 heat stressed. Results also showed a highly significant reduction in tetra-iodothyronine level (T4) in group T2 than all other groups, while increase in the control group. The previous results may be explained as there is a direct relationship between the degree of temperature used in heat stress and the decreased in tri-iodothyronine level in exposed birds.

Regarding corticosterone level, results cleared that there is a direct relationship between the degree of temperature used in the heat stress and the increased in corticosterone concentration in the exposed birds. These results may be explained as the level of corticosterone inversely related with thyroxin level, where the decreased T4 level increased secretion of cortisone. The level of corticosterone increased with the increased T3. These results are in consistent with. El-Nabarawy<sup>38</sup>, Galal<sup>39</sup>, Zaki<sup>35</sup> and Ghanem<sup>40</sup>, who reported that control group showed significantly lower (p<0.05) corticosterone concentration compared to heat stressed groups. Moreover, El-Bahy<sup>41</sup> explained the cause of decrease in T4 hormone level under heat stress, the high thermal stress will stimulates the hypothalamus to decrease the level of thyroid releasing hormone secretion which in turn affects on thyroid gland to decrease thyroid secretion. On the contrary to the previous results, Bowen and Washburn<sup>42</sup> and Yahav and McMurtry<sup>43</sup> found that plasma T3 concentrations were significantly reduced after thermal treatment, while T4 concentrations were increased.

#### CONCLUSION

Laying birds should be kept in a good thermal environment conditions. In the poultry house, if the environmental temperature is allowed to exceed normal ranges, especially when coupled with high humidity are extremely stressful for birds, which in turn affects laying metabolism which is responsible for egg production, quality and the overall performance.

#### SIGNIFICANCE STATEMENT

This study discovers the effects of thermal stress (temperature and relative humidity) which negatively affect poultry performance. This study will help the researcher to discover the best temperature humidity index (THI) level to decrease the deteriorating effect of thermal stress by using appropriate ways to relieve this stress in order to increase the production and decrease the economical losses.

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