

## Effect of Temperature-Humidity Index (THI) and Increased Heat Load on Rectal Temperature and Performance of Layer Hens Treated with Ascorbic and Folic Acids

<sup>1</sup>Okocha O.I. , <sup>1</sup>Okafor P.C., <sup>2</sup>Kanu C.N. and <sup>3</sup>Herbert U.

<sup>1</sup>Federal College of Education, Eha-Amufu, Enugu State, Nigeria. <sup>2</sup>Federal Polytechnic, Oko, Anambra State, Nigeria. <sup>3</sup>Michael Okpara University of Agriculture, Umudike, Abia State, Nigeria.

Corresponding author: [okocha.onuohaifeanyi@fceehamufu.edu.ng](mailto:okocha.onuohaifeanyi@fceehamufu.edu.ng)

### Abstract

This experiment was conducted to evaluate the effect of ascorbic acid and folic acid on management of heat load in the hot humid environment. Specifically, the investigation was to determine the interactional effect of temperature-humidity-index (THI) and increased heat load on the rectal temperature and performance of layer hens using ascorbic and folic acids as ameliorative agents. A total of 72 laying hens at 31 weeks of age were randomly divided into four (4) treatment groups of 18 birds per group and indicated as T1, T2, T3 and T4. All the treatment groups were subjected to 3-hours additional heat generated with a kerosene stove for 4-consecutive days of each week for 12 weeks. This additional heat was sufficient to induce intermittent panting and postural changes such as holding the wings out from the sides of the body. Diets for birds on T2, T3 and T4 were a layer's ration containing crude protein 16.5%, min. fat 5%, and maximum fiber 6%, max. calcium 3.50%, min. available phosphorus 0.41% and energy 2500kcal/kg supplemented with 250mg of ascorbic acid, 250mg of ascorbic acid+1mg of folic acid and 300mg ascorbic acid+1mg of folic acid respectively per Kg of feed. T1 served as the control group without supplementation. Records on THI, rectal temperature, daily egg production, daily feed intake, daily water consumption and body weight gain were examined. The results obtained showed that the control group recorded the highest significant ( $P < 0.05$ ) value of rectal temperature between treatment groups. The daily feed intake did not differ significantly ( $P > 0.05$ ) between treatments. The water consumption differed significantly ( $P < 0.05$ ) with the T1 consuming the highest volume of water per day. It was also observed that the Temperature-Humidity Index (THI) increased from 72.99 (week 2) to 73.14 (week12). These results suggest that combined supplementation of layer's diet with ascorbic acid (at 300mg/kg feed) and folic acid (at 1mg/kg feed) could be a nutritional management strategy in minimizing the negative impact of increased heat load coupled with very high relative humidity on rectal temperature and performance and hence is recommended for use in laying hens during the hot periods of the year.

**Keywords:** Layers, Heat Load, Ascorbic Acid, Folic Acid, Performance.

### Introduction

The Temperature Humidity Index (THI) is a measurement that combines temperature and humidity to determine the level of heat stress experienced by chickens. It takes into account

the impact of both variables on the bird's well-being. The THI is a crucial metric in chicken farming as it helps the farmers assess the environmental conditions in the pen. By understanding THI values, farmers can identify potential health and productivity issues that may arise due to extreme temperatures and humidity (Estrada Pareja, *et al.*, 2007).

Understanding and effectively managing the Temperature Humidity Index (THI) is essential for successful chicken farming. Farooq *et al.*, (2010) had stated that by considering THI values, implementing appropriate measures, and monitoring environmental conditions, a livestock farmer can create an optimal environment that promotes the health and well-being of the livestock including chickens and ultimately leading to improved productivity and profitability.

The chicken farmer can regularly monitor the vicissitudes of temperature humidity index (THI) using the rectal temperature of the chickens. The rectal temperature (RT) on the other hand, is recognized as an important measure of physiological status as well as ideal indicator for assessment of stress in animals (Ovaris and Anjali, 2016). Even a rise of less than 1°C in rectal temperature was enough to reduce performance in most livestock species (Ovaris and Anjali, 2016). RT is generally considered to be a useful measure of body temperature and changes in RT indicate changes of a similar magnitude in deep body temperature (Farooq, *et al.*, 2010) and (Ovaris and Anjali, 2016). The normal range in RT is very narrow in most domestic animals, not more than about 2.5°C (Ovaris and Anjali, 2016). The THI is a critical factor in assessing heat stress in poultry, including layer chickens. High THI values can negatively affect the bird's health and performance. THI is calculated using temperature and relative humidity, and it can impact the rectal temperature of layer chickens (St-Pierre *et al.*, 2003). Many researchers have confirmed that as THI increases, layer chickens may experience an increase in rectal temperature due to their limited ability to dissipate heat efficiently. High THI values can lead to reduced feed intake, lower egg production, and even mortality in severe cases (St-Pierre *et al.*, 2003).

### Statement of the Problem

In laying birds and all other farm animals, one of the most important stress factors from an economic standpoint is heat stress (Campbell *et al.*, 2003). Change in temperature has been considered a reliable indication of heat storage in animals' body and may be used to measure the severity of thermal environment, which can affect growth and other performance parameters (Karaman *et al.*, 2007). The high rectal temperature normally observed in the heat stressed animals is the indicator of disturbance in the homeothermic status of the animals which was not being effectively countered by the enhanced heat loss by physical and physiological processes of thermolysis (Estrada Pareja, *et al.*, 2007). However, during hot and especially humid conditions, the natural ability of birds to dissipate heat is compromised due to the lowered ability to utilize evaporative cooling (Tao and Xin, 2003).

The THI directly influences chicken health and behavior. High THI values can lead to heat stress, reduced feed intake, weight loss, lower egg production and increased mortality rates. Chickens may also exhibit panting, wing spreading, and reduced activity levels as coping mechanism. Although regular monitoring of THI levels using reliable weather sensors allows farmers to stay informed about the environmental conditions within the poultry pen, one of the reliable indicators of THI on the chicken is its rectal temperature. This enables the chicken farmer to make timely decisions and implement appropriate measures to ensure the well-being and productivity of their chickens.

High temperatures, especially when coupled with high humidity, impose severe stress on birds and lead to reduced performance (Joachim and Angel, 2011). Although a great deal of knowledge has been acquired concerning the responses of poultry to high ambient temperatures, the role of relative humidity (RH) in intensifying or modifying these responses has received little attention and more particularly air velocity (Simmons *et al.*, 2003). Relative humidity (RH) is rarely included as an experimental variable or even measured for information purposes. Such information is important because in poultry-producing regions, high temperatures can often be accompanied by a range of RH, which can markedly affect the degree of heat stress experienced by the birds (Balanve, 2004). During heat stress, the environmental parameters of ambient temperature (AT) and relative humidity (RH) in general and temperature humidity index (THI) in particular, have been reported to be an invaluable tool in the presumptive diagnosis of the animal state of health, and is also relevant in evaluating the adaptability of the animal (Tao and Xin, 2003).

### Research Questions

The following research questions guided the study;

1. What is the Temperature Humidity Index (THI) of the experimental compartment during increased environmental heat load?
2. What is the effect of THI on rectal temperature of layer chickens treated with ascorbic and folic acids during increased environmental heat load?
3. What is the effect of THI on performance (i.e. daily egg production, daily feed intake, daily water consumption and daily body weight gain) of layer chickens treated with ascorbic and folic acids during increased environmental heat load?

### Objectives of the Study

The broad objective of the study was to determine effect of temperature-humidity index (THI) and increased heat load on rectal temperature and performance of layer hens treated with ascorbic and folic acids. The specific objectives are to determine;

1. The Temperature Humidity Index (THI) of the experimental compartment during increased environmental heat load.
2. The effect of THI on rectal temperature of layer chickens treated with ascorbic and folic acids during increased environmental heat load.

3. The effect of THI on performance (i.e. daily egg production, daily feed intake, daily water consumption and daily body weight gain) of layer chickens treated with ascorbic and folic acids during increased environmental heat load.

### Literature Review

Birds, during heat-stress, have a greater challenge in maintaining homeothermic body temperature (41.5°C) compared to other domestic animals (Cifti, Nihat-Ertas and Guler, 2005, Sahin *et al.*, 2009). Above 24°C, the bird has a number of possibilities to remove the excessive body heat. An increased environmental heat load can significantly affect a chicken's rectal temperature. Chickens are homeothermic animals, meaning they maintain a relatively constant body temperature (Talebi and Khademi, 2011). When exposed to high environmental temperatures, several physiological responses occur to dissipate heat and regulate their temperature. These include panting, reduced feeding, vasodilation and reduced activity. Despite these mechanisms, if the environmental heat load becomes too extreme, chickens can experience heat stress. This can result in a significant increase in rectal temperature. Prolonged exposure to high temperatures can lead to dehydration, heat stroke, and even death. It's essential to provide chickens with shade, adequate ventilation, and access to cool, clean water to help them cope with increased heat load and maintain their rectal temperature within a healthy range.

The body temperature of a broiler chicken varies between 40°C and 42°C (DEFRA, 2006) depending on day time (before and after feeding, night time), feather cover in connection with molting, brooding and environmental temperature. For poultry, the thermo-neutral zones i.e. the ideal environmental temperatures ranges from 18 to 22°C. Within this zone, animals are expected to be comfortable, showing no problems associated with physical temperature regulation (DEFRA (2006) and Sahin *et al.*, 2009).

With increase in environmental temperature, there is an enlarged dependence on insensible heat loss for maintaining body temperature. With an environmental temperature of 26°C, causing a rise of 0.1 – 0.4°C in body temperature above the normal, the rise in the temperature of the blood flowing to the brain initiates panting (Frandsen, *et al.*, 2009). This in turn causes a dramatic rise from 5g to 30g per hour in water loss through evaporation. Beyond 32°C environmental temperature, however, there seems to be a breakdown in thermo-regulation as respiration rate rises to a maximum of 140°C and then starts to decline (Frandsen, *et al.*, 2009).

### Materials and Methods

#### Experimental Site and Duration

The experiment was carried out at the Poultry Unit of the Teaching and Research Farm of Michael Okpara University of Agriculture, Umudike, MOUAU, Abia State, Nigeria. The University is located on an elevation of about 120m above sea level at latitude 5°21' North and longitude 7°29' East. Umudike falls within the rainforest zone of Nigeria which is

characterized by hot and humid climate. The mean annual rainfall is about 90% and that of temperature is 22° to 36°C depending on the season (N.R.C.R.I, 2013). There has been consistent increase in the environmental temperature with an accompanying slight increase in relative humidity of the ecological zone in recent time mostly due to the global climatic warming syndrome. The experiment lasted for a period of 14 weeks between February and May, 2014.

### Experimental Animals and Management

A total of 72 layers aged 31-weeks were used and were purchased from the commercial poultry farm of MOUUAU Nigeria Ltd, Umudike. The laying birds were managed under deep-litter intensive system. The poultry pens were naturally cross-ventilated and the birds were screened and observed for a period of 2-weeks before the commencement of the experiment to prevent external environmental influence and allow the animals acclimatize their new environment following which heat treatment and data collection commenced while maintaining a clean environment.

The birds were grouped into 4 treatments of 18 birds per treatment indicated as T<sub>1</sub> (Treatment 1), T<sub>2</sub> (Treatment 2), T<sub>3</sub> (Treatment 3) and T<sub>4</sub> (Treatment 4). Treatment 1 served as the control. Routine medications were strictly observed during the experiment.

### Experimental Design

The birds were fed layer's ration containing crude protein 16.5%, min. fat 5%, max. Fiber 6%, max. calcium 3.50%, available phosphorous 0.41% and energy 2500kcal/kg and supplemented as shown in the Table 1.

**Table 1.** Experimental Design

Treatment Group	Treatment Specification
Treatment 1 (T <sub>1</sub> )	3-hours duration of additional heat/day, not supplemented commercial layers' mash.
Treatment 2 (T <sub>2</sub> )	3-hours duration of additional heat/day and a commercial layers' mash supplemented with 250mg of ascorbic acid per kg of feed
Treatment 3 (T <sub>3</sub> )	3-hours duration of additional heat/day and a commercial layers' mash supplemented with 250mg of ascorbic acid + 1mg of folic acid per kg of feed
Treatment 4 (T <sub>4</sub> )	3-hours duration of additional heat/day and a commercial layers' mash supplemented with 300mg of ascorbic acid + 1mg of folic acid per kg of feed.

Feed and water were served in all the treatment groups ad libitum.

The study was a Completely Randomized Design (CRD) with four (4) treatments and 3 replicates per treatment.

The statistical model was as follows

$$Y_{ij} = \mu + T_i + e_{ij}$$

Where;

$Y_{ij}$  = single observation     $T_i$  = Treatment effect

$\mu$  = overall mean                       $e_{ij}$  = Random error

### **Experimental Procedures and Data Collection**

#### **Ambient Temperature-Relative Humidity Index and Rectal/Body Temperature**

A standard thermometer (Harris, England) with a calibration of  $42^{\circ}\text{C}$  was used to measure the ambient temperature of the experimental compartment. Both the initial and final room temperatures were recorded on a daily basis throughout the duration of the experiment. A kerosene stove was used to generate additional heat which was sufficient to induce intermittent panting and postural changes such as holding the wings out from the sides of the body. The intention was to stimulate a degree of heat stress which is experienced commonly by commercial layer chickens. This heat stress inducement was done for all the treatments groups during the course of the experiment for four consecutive days of each week.

The additional heat generated was observed using the simple reading of the thermometer. This additional heat maintained the temperature of the experimental compartments at  $34^{\circ}\text{C}$  for 3-hours during each 4-consecutive days of each week and this heat treatment lasted for ten (12) weeks during which data were collected.

Also, a standard hygrometer (Cocet, China) with a  $50^{\circ}\text{C}$  calibration was used to record the daily relative humidity (RH). A clinical digital thermometer was inserted below the tongue to determine body temperature and also inserted into the rectum to determine the rectal temperature of the experimental animals daily.

The rectal temperature was taken at 7.00am, 12noon and 5pm during each of the 4-consecutive days of heat treatment. The THI was subsequently determined.

### **Measurement of Other Parameters**

#### **Body Weight**

The body weight of individual birds within each treatment group was taken at the commencement of the experiment and subsequently on weekly basis using a sensitive balance while the values were recorded accordingly to determine their growth performance.

#### **Feed and Water Consumption**

The daily feed intake was recorded by weighing the feed given to the animals and leftover. The feeding trough was placed in such a way that wastage by the animals was prevented and feed was usually served before 9.00am daily.

Although water was given ad libitum, the water consumed by the animals was determined by weighing the volume given and weighing the volume withdrawn under the assumption of constancy in environmental conditions. The water given was normally within room temperature and to achieve this to a reasonable extent, the water in the water trough was changed or replaced in the mid-afternoon to enhance water consumption.

### Statistical Analysis

All data were subjected to ANOVA according to the procedures described by Steel and Torrie, (1980) as described by Okocha and Herbert, (2022). Significant treatment means were separated using the least significant difference (LSD).

## Results and Discussion

### Temperature-Humidity Index (THI)

The temperature-humidity index data is presented in Table 2.

**Table 2.** Temperature-Humidity index during the experiment

Week	2	4	6	8	10	12	SEM
THI	72.99 <sup>c</sup>	73.06 <sup>c</sup>	73.09 <sup>b</sup>	73.12 <sup>ab</sup>	73.11 <sup>b</sup>	73.14 <sup>a</sup>	0.01

**Source:** Experimentation, 2022

a,b,c,- means with different alphabetical superscripts across the rows are significantly different at  $P < 0.05$ ; SEM- Standard error of the mean, THI- Temperature Humidity Index. The values of THI recorded in this study are above the threshold mark of 70 established for poultry by Tao and Xin (2003). This indicates that the high ambient temperature (AT) and high relative humidity (RH) acting on the birds during the period predisposed the birds to heat stress, as evidenced by a decrease in production parameters. This finding is in agreement with the report by Karaman *et al.*, (2007), who reported a decrease in egg production and feed intake.

Also, as the THI values progressively increased in the course of the experiment, there was a numerical upward increase of the rectal temperature within each treatment group. This was in agreement with the findings of Tao and Xin (2003) who reported a positive relationship between THI and rectal temperature. The chain effects of THI on rectal temperature is being propelled by the elevation of the respiratory rate (panting condition) and water intake as reported by Farooq *et al.*, (2010) and depression of appetite center (due to a direct negative effect on appetite center of the hypothalamus) as reported by Frandson *et al.*, (2009).

This positive relationship between THI and rectal temperature is in agreement with the report of Estrada-Pareja *et al.*, (2007) and Tao and Xin (2003), who stated that high temperatures, especially when coupled with high humidity, imposes severe stress on birds and leads to reduced performance.

### Rectal Temperature

The results of the rectal temperature of layer hens exposed to increased heat load in hot humid environment treated with ascorbic and folic acids are presented in Table 3.

**Table 3.** Rectal Temperature of Layer Hens exposed to increased heat load in hot humid environment treated with ascorbic and folic acids

Weeks	T <sub>1</sub>	T <sub>2</sub>	T <sub>3</sub>	T <sub>4</sub>	SEM
2	31.01	30.74	30.45	30.32	0.15
4	30.87	31.54	31.91	31.81	0.44
6	36.41	36.88	36.97	36.66	0.70
8	40.67 <sup>a</sup>	40.11 <sup>ab</sup>	40.15 <sup>ab</sup>	40.04 <sup>b</sup>	0.10
10	40.53 <sup>a</sup>	40.03 <sup>ab</sup>	39.39 <sup>b</sup>	39.25 <sup>b</sup>	0.16
12	40.09	39.89	39.94	39.44	0.12

**Source:** Experimentation, 2022

a,b,c,- means with different alphabetical superscripts across the rows are significantly different at  $P < 0.05$ ; SEM- Standard error of the mean.

There was no significant difference ( $P > 0.05$ ) across T<sub>1</sub>, T<sub>2</sub>, T<sub>3</sub> and T<sub>4</sub> from week 2 up to week 6, indicating a non-significantly different positive impact of supplementing ascorbic and folic acids at different levels of inclusion. This trend also suggests that it probably could have taken up to the 6<sup>th</sup> week for the single or combined dietary supplementation of ascorbic and folic acids to establish its significant influence on rectal temperature of heat stressed layers in hot humid environment.

However, T<sub>1</sub> (the control group) recorded the highest value of rectal temperature and showed the highest significant value from week 8 to week 12. There was also a declining variation among the treated groups with T<sub>4</sub> having the least significantly different rectal temperature values from week 8 to week 12. This result confirms the findings of Altan *et al.*, (2003) who reported that high ambient temperature and relative humidity increases heat stress and are responsible for the increase in rectal/body temperature. Also, the higher rectal temperatures observed in the non-supplemented group (T<sub>1</sub>) down the course of the experiment is the indicator of disturbance in the homeothermic status of the animals which was not being effectively countered by enhanced heat loss response initiated by the presence of anti-oxidants such as ascorbic acid.

### Performance Parameters

Table 4 presents the performance characteristics of layer hens exposed to increased heat load in hot humid environment treated with ascorbic and folic acids.



**Table 4.** Performance Characteristics of Layer Hens exposed to increased heat load in hot humid environment treated with ascorbic and folic acids

Parameters	T <sub>1</sub>	T <sub>2</sub>	T <sub>3</sub>	T <sub>4</sub>	SEM
Daily Egg Production/trt	6.90 <sup>b</sup>	7.56 <sup>ab</sup>	8.15 <sup>a</sup>	8.04 <sup>a</sup>	0.14
Daily Feed intake (Kg/bird)	0.19	0.21	0.21	0.20	0.04
Daily Water Cons.(ltr/bird)	0.36 <sup>a</sup>	0.34 <sup>b</sup>	0.32 <sup>bc</sup>	0.31 <sup>c</sup>	0.05
Init. Body weight (g/bird)	1651.45	1650.14	1654.82	1647.38	1.81
Final Body weight (g/bird)	1670.67 <sup>c</sup>	1672.18 <sup>b</sup>	1679.00 <sup>a</sup>	1672.81 <sup>b</sup>	1.97
Body weight gain (g/bird)	19.22 <sup>c</sup>	22.04 <sup>c</sup>	24.18 <sup>b</sup>	25.43 <sup>a</sup>	1.39

Experimentation, 2022

a,b,c,- means with different alphabetical superscripts across the rows are significantly different at  $P < 0.05$ ; SEM- Standard error of the mean.

### Daily Egg Production

The effect of ascorbic acid and folic acid supplementation on daily egg production of heat-stressed layers in hot humid environment was significant ( $P < 0.05$ ). The combined inclusion of ascorbic and folic acids at the ratio of 250mg:1mg respectively per Kilogram of feed (T<sub>3</sub>), recorded the highest mean value (8.15) followed by T<sub>4</sub> (300mg:1mg). Lowest daily egg production was recorded in untreated control group (T<sub>1</sub>-6.90). This result is in agreement with the previous findings of Khan and Sardar (2005), who reported that vitamin C supplementation in layer birds improved significantly the mean egg production, egg weight and egg shell thickness.

### Daily Feed Intake

The daily feed intake values were not significantly different ( $P > 0.05$ ) but recorded the highest value in T<sub>1</sub> followed by T<sub>3</sub>, T<sub>4</sub> and T<sub>1</sub> in descending order. This non-significantly different positive impact of single and combined dietary supplementation of ascorbic and folic acids on feed intake of this experiment numerically corroborates with the findings of Khan and Sardar, (2005), that the single or combined dietary supplementation with vitamin C and vitamin E of laying chickens exposed to heat stress significantly improved production performance of feed consumption, conversion and egg/bird/day while Holik, (2009) reported that chickens usually withdraw from feed when exposed to extreme ambient temperature.

The result of this study further explains that the laying chickens in the treated groups (T<sub>2</sub>, T<sub>3</sub> and T<sub>4</sub>) must have been able to harness the antioxidant effect of vitamin C in neutralization of the free radicals generated during heat stress.

### Daily Water Consumption

The daily water consumption per liter per treatment group was significant ( $P < 0.05$ ). The birds in the control group consumed the highest volume of water per day on the average

(6.47ltr/bird), indicating higher penchant for water. This observation is explained as a defensive or survival mechanism employed by heat-stressed layers that usually elects to pant. This scenario is further explained by Campbell *et al.*, (2003) who reported that panting expends large amount of water, which must be replaced if the animal is to maintain effective heat regulation.

The lower mean values of T<sub>2</sub> (6.04ltr/bird), T<sub>3</sub>(5.77ltr/bird) and T<sub>4</sub> (5.65ltr/bird) strongly suggest that single or combined supplementation with vitamin C and folic acid will significantly depress or reduce the rush to water by heat-stressed laying birds of hot humid environment.

### Body Weight Gain

Combined supplementation of ascorbic and folic acids at the ratios of (250mg:1mg) and (300mg:1mg) i.e. T<sub>3</sub> and T<sub>4</sub> respectively had numerically higher significant values ( $P < 0.05$ ) than that of control group and the T<sub>2</sub> (single ascorbic acid supplementation). The combined supplementation of ascorbic acid and other coenzymes such as folic acid has been scientifically well proven to increase body weight gain as well as improve growth and performance of birds during heat stress (Sahin *et al.*, 2003).

### Conclusion

It is concluded that the negative impact of increasing Temperature-Humidity-Index (THI) on rectal temperature and performance, significantly reduced due to single or combined supplementation of ascorbic and folic acids.

### Recommendations

Based on the findings of this study, the following recommendations are made:

- Oral administration of 250mg of ascorbic acid and 1mg of folic acid should be used in the management of increased environmental heat load of layer chickens in hot humid environment.
- During the seasons of high temperatures coupled with high relative humidity, heavy or severe panting could be prevented by the oral administration of 250mg of ascorbic acid and 1mg of folic acid.
- Oral administration of 250mg of ascorbic acid and 1mg of folic acid can be used to improve daily egg production and daily weight gain of layer chickens exposed to increased environmental heat load.

### References

- Altan, O, Pabuccuoglu A, Konyalioglu, S, and Bayraktar, H (2003). Effect of heat stress on oxidative stress, lipid peroxidation and some stress parameters in broilers. *Poult Sci* 2003; 44(4):545-550.
- Balnave, D, (2004) Challenges of accurately defining the nutrient requirements of heat-stressed poultry. In: World's Poultry Science Association Invited Lecture. *Poult Sci*; 83:5-14.

- Cifti, M., Nihat-Ertas, O. and Guler, T. (2005). Effects of vitamin C dietary supplementation on egg production and egg quality of laying hens exposed to a chronic heat stress. *Revue. Med. Vet.* 156(2):107-111.
- Campbell, J.R., Kenealy M.D. and Campbell, K.L. (2003): *Animal Sciences (The Biology, Care and Production of Domestic Animals)*. 4<sup>th</sup> edn. McGrawHills, New York pp. 77.
- DEFRA (2006). Department For Environment, Food and rural Affairs, Botswana.
- Estrada-Pareja, M.M, Marquez-Giron, S.M, and Restrepo, L.F, (2007). Effect of temperature and relative humidity on productive parameters and heat transfer in broiler chickens. *Rev Col Cienc Pec*; 20:288-303.
- Farooq, U, Samad, H.A. Shehzad, F. and Qayyum, A. (2010). Physiological Responses of Cattle to heat stress. *World Applied Sciences Journal (Special Issue of Biotechnology and Genetic Engineering)*:38-43.
- Frandsen, R.D., Wilke, W.L. and Fails, A.D. (2009). *Anatomy and Physiology of Farm Animals* (7<sup>th</sup> edn.) Wiley-Blackwell Publishing, U.S.A.
- Holik, V. (2009). Management of laying hens to minimize heat stress. *Lohmann Information*; 44(1):16-29.
- Joachim, J.A. and Angel, M.T. (2011). Impact of Heat-Stress on egg quality in layer hens supplemented with L-ascorbic acid and dl-tocopherol acetate. *Vet. Arch.* 81(1):119–132
- Karaman, S, Tarhan, S, and Ergunes G, (2007). Analysis of Indoor Climatic Data to Assess the Heat Stress of Laying Hens. *Int. J. Nat. Engl. Sci.* (Suppl II): 65–68.
- Khan, S.H, and Sardar, R. (2005). Effect of Vitamin C Supplementation on the Performance of desi, faoumi and commercial white leghorn chicken exposed to heat stress. *Pak. Vet. J.* 25(4):136-166.
- NRCRI (2013) National Root Crops Research Insitute, Umudike Weather Station Report 2013.
- Okocha, O.I. and Herbert, U. (2022). Effect of vitamin C and folate on heat-stressed chickens' egg quality and daily egg production. *International Research Journal of Science, Technology, Education and Management.* 2(2), 204-215. <https://doi.org/10.5281/zenodo.6973543> Available online at <https://irjstem.com>
- Ovaris, A. and Anjali, A. (2016). Dry period cooling ameliorates physiological variables and bllod acid milk production in murrah buffaloes. *Int. J. of Biometeorol.* 60(3):465-75 <https://doi:10.1007/s00484-0151044-4>
- Sahin, K., Onderci, M., Sahin, N, Gursu, M.F. and Kucuck, O. (2003). Dietary Vitamin C and folic acid supplementation ameliorates the detrimental effects of heat stress in Japanese quail. *Journal of Nutrition* 133:1882-1886.
- Simmons, J.D, Lott, B.D, and Miles, D.M, (2003). The effect of High-Air Velocity on Broiler Performance. *Poult. Sci.* 82: 232-235.
- St-Pierre, N.R., Cobanov, B. and Schnitkey, G. (2003). Economic losses from heat stress by US Livestock Industries. *Journal of Dairy Science* 86:(E.spl.) E52-E77.
- Tao, X, and Xin, H, (2003). Temperature-humidity-velocity-index for market size broilers. Nevada, USA: *Proceedings of the ASAE Annual International Meeting*; 2003. pp. 54.
- Talebi, E., Khademi, M. (2011). Combination Effects of Ascorbic Acid and Glucose in Drinking Water on the broiler performance under Acute heat Stress. *International Journal of Applied Biology and Pharmaceutical technology*, 2(1):92-6.