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Effect of vitamin C and folate on heat-stressed chickens' egg quality and daily egg production

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ABSTRACT

Egg quality is of economic consideration especially in thermally challenged confinements during table egg production and transportation. The effect of vitamin-C and folate on heat-stressed chickens' egg quality and performance is aimed at overcoming the negative impact of exposure to increased environmental heat load at peak heat periods of the year using vitamin-C as ameliorative agent, and efficient utilization of consumed feed, using folate. This is in other to lay quality eggs and perform optimally. A total of 72 Isa Brown laying hens at 31 weeks of age were randomly divided into 4 treatment groups of 36 birds subdivided into 3 replicates and indicated as T1, T2, T3 and T4 while T1 served as the control treatment. The birds were housed in a deep litter pen and exposed to 3-hours additional heat generated with gas-powered burners, for four consecutive days of each week for a period of 12 weeks. The diets for T2, T3 and T4 were supplemented with 250 mg of vitamin-C, 250 mg of vitamin-C + 1 mg of folate and 300 mg of vitamin-C + 1 mg of folate per kg of feed respectively. The results showed that T4 had the highest egg weights (60.04 g), shell thickness (0.53 mm) and shell weights (7.80 g). The albumin weight (37.20 g), albumin height (6.80 mm) and yolk weight (14.70 g) were highest ($P<0.05$) in T4 while daily eggs production/treatment/day was highest in T4 (8.04). The daily feed intake was ($P<0.05$) highest in T3 and T4 (0.42 kg/bird) and ($P<0.05$) lowest in T3 and T4 (1.51). It is concluded that combined supplementation of layers' diet with vitamin-C and folate at the ratio of 350 mg: 1 mg/kg of feed) help to reduce negative impact of heat stress and enhance efficient utilization of feed consumed. It is recommended as a nutritional management strategy in minimizing the negative impact of increased heat load coupled with very high relative humidity.

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INTRODUCTION

One known cause of egg quality and egg shell quality problems which occur commonly in hot humid environments is heat stress. It is usually caused by high ambient temperature (David et al., 2012). When increased environmental temperature is combined with high humidity, it usually imposes severe stress on laying chickens and leads to reduced performance (Joachim and Angels, 2011). The reduction in reproductive performance such as the egg-laying parameters associated with heat stress is a well-known phenomenon in domestic birds (Rozenberg et al., 2007). This could possibly be due to a reduction in ovarian blood flow because a differential ovarian blood flow pattern was found in laying hens exposed to high ambient temperatures (Joubrane et al., 2019). The response of chickens at high temperatures differs with relative humidity.

In the tropical wet climates and particularly in the tropical humid environments of Nigeria, assisting layer chickens adjust to the negative impacts of increased heat load has remained a topical issue. In the last two decades, there has also been a great deal of research and development in ways and means of reducing heat stress in birds subjected to high temperatures (Nadia et al., 2021). The Isa Brown layer chicken is a crossbreed of chicken with sex-linked coloration. It is known for its high egg production of approximately 300 eggs per hen in its first year of laying. It is able to adapt well to different climates, poultry management styles and housing systems and is the commonest available commercial chicken layer strain in the warm wet West African Sub region for the production of brown table eggs (HENDRIX GENETICS, 2022). Although poultries particularly are renal synthesizers of vitamin C, their quantity becomes insufficient during increased heat load as a result of the increased rate of usage in combating the free radicals thus generated (Friday et al., 2021). Arguably, with enhanced nutrient utilization due to folate supplementation, the effect of vitamin-C stands better improved predominantly in the wake of the recent increasing threat of global climatic warming. This effect of climate change in the world has necessitated this study. Many authors have recommended the supplementation of multivitamins and minerals, especially in layer chickens (Nadia et al., 2021; Joubrane et al., 2019; Yakubu et al., 2018). Nadia et al., (2021) have recommended ascorbic acid (vitamin-C) in drinking water or feed during hot period of the day. Friday et al., (2021) had also reported that vitamin-C has antioxidant properties which neutralizes the free radicals generated during heat stress while folate (folic acid) has been found to enhance nutrient utilization by poultry species. Folates had been reported to be involved in inter conversion of amino acids, such as serine and glycine, and for the synthesis of methionine from homocysteine (Bagheri et al., 2018). Animals are not renal synthesizers of folates but are required to process efficient intestinal absorption, after which all shapes of folates are delivered through hepatic portal system to liver (Bai et al., 2021). The folate requirement of laying hens was reported by the National Research Council (1994), which were 0.25 mg/folate/kg of feed. Toghyani et al., (2022) reported that folate supplementation improved feed efficiency over the entire production cycle of laying hens under a long term production condition. Bagheri et al., (2018) also observed that increasing dietary folate in diet improved feed conversion ratio (FCR) and hen day egg production.

The results obtained from these investigations have suggested early acclimatization (Cayh et al., 2021), nutritional manipulation and improved modern housing (Shakeri et al., 2020). Housing and ventilation equipment are two of the most effective means of reducing heat stress though, the cost of building and maintenance is too high. This has led to seeking alternative cost-effective ways of combating heat stress without creating additional stress in birds especially when combined with high humidity. The need for thermally-challenged layer chickens to lay quality eggs remains an economic standpoint. This is especially necessary in economically poor tropical humid environments, where egg handling and transportation is mostly poorly managed. Assisting layer chickens exposed to increased environmental heat load at peak heat periods of the year (usually between months of January and March); using vitamin-C and enabling them to effectively utilize consumed feed during heat stress, using folate in other to lay quality eggs and perform optimally; solves an economic problem for the poultry industry. Therefore, there is also a research need to conduct some relevant examinations to meet nutritional requirements of folate in industrial poultry strains such as the commonly available Isa Brown layer strains; with improved production traits. According to Satar et al., (2019), the improved production traits in new strains of poultry, present a higher folate requirement above the recommendation by National Research Council of the United States of America (NRC,

1994) for laying poultry (0.25 mg/kg diet). Due to the high nutritional value of poultry table egg and its comparatively low price to other animal products, they are highly indicated to alleviate nutritional problems in low-income populations. However, in order to improve their accessibility to larger populations, there is a need to increase quality indexes related to their handling, transportation, and preservation.

OBJECTIVES

The general objective of the study is to assess the influence of vitamin C and folate as ameliorative agents against the negative effects of increased heat load on egg quality and performance of laying chickens. The specific objectives are:

1. estimate the external egg quality parameters of heat-stressed layer chickens supplemented with vitamin C and folate in deep litter poultry pens during peak heat periods of the year.
2. estimate the internal egg quality parameters of heat-stressed layer chickens supplemented with vitamin C and folate in deep litter poultry pens during peak heat periods of the year.
3. examine the interaction effect of vitamin-C and folate on performance characteristics of heat-stressed layer chickens in deep litter poultry pens during peak heat periods of the year.

METHODS

Design

The experiment was carried out in a completely randomized design (CRD) with four (4) treatments. The treatment consisted of 3-hours duration of heat application and vitamin C and folates supplementation as specified in treatment specifications.

The statistical model is as follows:

$$Yij = \mu + Ti + eij$$

Where Yij = Single observation
μ = overall mean
Ti = Treatment mean
eij = Random error

Study area

The experiment was carried out at the Poultry Unit of the Teaching and Research Farm of Michael Okpara University of Agriculture, Umudike, Umuahia, Abia State, Nigeria. The University is located on an elevation of about 120 m above sea level at latitude 5^{o}21'North and longitude 7^{o}29' East. Umudike falls within the rainforest zone of Nigeria which is characterized by a hot and humid climate. The mean annual rainfall is between 2,500 mm to 3,000 mm and that of temperature is 22^{o}C to 36^{o}C (N.R.C.R.I., 2014). The experiment lasted for a period of 14 weeks between January and April, 2014.

Experimental animals and management

A total of 72 Isa Brown layer chickens aged 31 weeks, purchased from the commercial poultry farm of Michael Okpara University of Agriculture, Umudike Nigeria Ltd, were used for the study. The laying birds were managed under a 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 influences. This was to allow the animals to acclimatize to their new environment, following which heat treatment, experimental diet administration, and data collection commenced while maintaining a hygienically clean environment. The birds were divided into 4 groups of 18 birds per treatment indicated as T1 (Treatment 1), T2 (Treatment 2), T3 (Treatment 3), and T4 (Treatment 4) while each treatment group was further replicated 3 times. Routine prophylactic hygienic measures were strictly observed during the experiment.

Experimental design

The birds were fed layer chickens' ration containing crude protein 16.5%, 2500 kcal/kg and supplemented as shown in Table 1.

Table 1. Experimental design

Treatment Group	Treatment Specification
Treatment 1 (T1)	3-hours duration of additional heat/day, not supplemented commercial layers' mash.
Treatment 2 (T2)	3-hours duration of additional heat/day and a commercial layers' mash supplemented with 250 mg of ascorbic acid per kg of feed
Treatment 3 (T3)	3-hours duration of additional heat/day and a commercial layers' mash supplemented with 250 mg of ascorbic acid + 1 mg of folic acid per kg of feed
Treatment 4 (T4)	3-hours duration of additional heat/day and a commercial layers' mash supplemented with 300 mg of ascorbic acid + 1 mg of folic acid per kg of feed.

Adopted from Okocha and Herbert, (2016)

Feed and water were served in all the treatment groups ad libitum. The study was a Completely Randomized Design (CRD) with 4 treatments and 3 replicates per treatment.

Ethical consideration

All the protocols regarding animal welfare were observed in handling the experimental animals.

Experimental procedures and data collection

Increment in environmental heat load:

A cooking gas-powered 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 especially during peak heat periods between months of January and April. This heat stress inducement was done for all the treatments groups during the course of the experiment for 4 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 between 34^{o}C to 40^{o}C for 3-hours during each 4-consecutive days of each week and this heat treatment lasted for 11 weeks during which data were collected.

Egg production and egg quality parameters

The following parameters were measured on egg and the average record taken weekly. 6 randomly selected eggs were used from each treatment per week for egg analysis and the average obtained.

Egg production: Eggs were collected daily at 09:00h and weighed per replicate. Hen-day egg production was calculated as the total eggs laid/hen-days multiplied by 100. Eggs (n=6/replicate) were collected on the 4 consecutive days of heat treatment and egg quality measured. *Egg weight (g):* The average weight of all eggs laid by all the birds in each treatment was recorded daily using an electronic scale. *Shell thickness (mm):* The average shell thickness of all the eggs laid in each treatment was recorded using a micrometer screw gauge. *Egg length (mm) and Egg width (mm):* The average egg length and egg width of each treatment was determined using a micrometer pointer. *Shell weight (mm):* The average weight of the eggs laid by each treatment group was recorded with an electronic scale. This was done after carefully breaking and emptying of the egg content. The shell was weighed together with the outer shell membrane intact. *Internal egg parameters:* The internal egg quality was assessed by measuring the Haugh Units of the albumen. The egg was weighed, then broken onto a flat surface (breakout method) and a micrometer used to determine the height of thick albumen. *Albumen weight (g) and yolk weight (g):* Both the albumen weight and yolk weight were measured with an electronic scale using a petri-dish as described by Leeson, (2006). *Albumen height (mm) and albumen diameter (mm):* The average albumen height and albumen diameter were recorded using a micrometer pointer as described by Leeson, (2006). *Yolk height (mm) and yolk diameter (mm):* The average yolk height and yolk diameter were recorded using a micrometer pointer as described by Leeson, (2006). *Haugh unit (HU):* The Haugh unit was estimated using the equation according to (Haugh, 1937 in Joubrane *et al*., 2019).

$HU = 100\log (H + 7.57 - 1.7W^{0.37})$
HU= Haugh Unit
H= observed albumin height (mm)
W= observed weight of egg (g)

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. *Feed Conversion Ratio (FCR):* The feed conversion ratio was expressed as kg of feed consumed per kg of eggs produced and kg of feed consumed per 12 eggs laid. *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.

Statistical analysis

All data were subjected to one way analysis of variance (ANOVA) according to the procedures described by Steel and Torrie, (1980) and reported by Kayomba et al., (2022). The significant treatment means were separated using the least significant difference (LSD).

Week 1 Activities:

***Cleaning and disinfection of deep-litter poultry pens**

***Random allotment of layer chickens into treatment groups and administration of anti-stress**

↓

Week 2 Activities:

***Deworming and administration of broad spectrum antibiotics**

***Plain water administration**

↓

Week 3 Activities:

***Vaccination against New castle disease and Infectious bursa disease**

***Plain water administration and weighing of birds per treatment**

↓

Week 4 – Week 14 Daily Routine Activities:

***Administration of experimental diets from beginning of 4 to the end of week 14**

***Daily recording of average feed consumption per treatment**

***Daily recording of average water consumption per treatment**

*** Daily recording of average birds' weight/treatment**

*** 3-hour heating of experimental compartments to ambient temperature of 34-40°C between 9:00 am – 12:00 noon from Mondays-Thursdays from beginning of week 4 to the end of week 14.**

*** Collection and assessment of 2 eggs per replicate for external/internal egg quality parameters between 9:00 am – 12:00 noon from the beginning of week 4 to the end of week 14.**

↓

Week 15 Activities:

***Data collation and analysis**

Figure 1. Methodological framework of the experiment

RESULTS AND DISCUSSIONS

The results of the external egg quality parameters of heat-stressed layer chickens in hot humid environment treated with vitamin C and folates are presented in Table 2.

Table 2. External egg quality parameters of heat-stressed layers in hot humid environment treated with vitamin-C and folates.

Parameters	T1	T2	T3	T4	SEM
Egg weight (g)	57.11^{d}	58.61^{c}	59.78^{b}	60.83^{a}	0.48
Shell weight (g)	7.14^{b}	7.28^{b}	7.39^{ab}	7.80^{a}	0.08
Egg length (mm)	55.13^{d}	55.25^{c}	56.18^{a}	55.91^{b}	0.22
Egg diameter (mm)	43.78^{a}	43.74^{ab}	43.87^{c}	43.20^{b}	0.20
Shell thickness (mm)	0.47^{b}	0.47^{b}	0.49^{ab}	0.53^{a}	0.01

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

The external egg quality parameters are presented in Table 2. T4 recorded the highest mean values (P<0.05) of egg weight (60.04g), shell weight (7.80 g) and shell thickness (0.53 mm) while T1 recorded the least. The significant (P<0.05) reduction in shell weight, shell thickness in T1 due to the inducement of heat stress as a result of increased heat load confirms the findings of Roberts and Bald (1998). This reduction is further explained by Santin (2018), who stated that at high temperatures, birds pant to enhance evaporative cooling. These panting results in respiratory alkalosis which is caused by loss of CO_2 from the blood and involves an increase in blood pH which in turn decreases the proportion of the blood calcium that is in ionized form and thus, reduces the amount of calcium which is available for egg shell formation. When egg shells are thicker and heavier in weights, the shell strength is higher, crashed egg percentage is reduced during egg lay, handling and transportation and so, are able to be preserved till consumption. Eggs with thick and strong shells are usually the most marketable (Wasti et al., 2020). The higher egg weight may be due to efficient utilization of nutrients by the presence of folate, which enhanced albumin deposition as Saksrithai and King (2022) reported that albumin deposition is greatly affected by level of dietary protein. This may be an indication of superior ability of T4 in enhancing deposition of albumin during increased environmental heat load. The results of the internal egg quality parameters of heat-stressed layers in hot humid environment treated with vitamin C and folates are presented in Table 3.

Table 3. Internal egg quality parameters of heat-stressed layers in hot humid environment treated with vitamin-C and folates.

Parameters	T1	T2	T3	T4	SEM
Albumin weight (g)	34.47^{d}	34.50^{c}	36.67^{b}	37.20^{a}	0.50
Albumin height (mm)	6.15^{d}	6.36^{c}	6.63^{b}	6.80^{a}	0.13
Albumin diameter (mm)	60.78^{c}	61.40^{b}	56.87^{a}	57.22^{ab}	1.03
Yolk weight (g)	13.90^{b}	14.47^{ab}	14.47^{ab}	14.70^{a}	0.20
Yolk height (mm)	15.08	15.08	15.01	15.07	0.13

Yolk diameter (mm)	36.12	36.26	36.08	38.15	0.37
Haugh Unit	77.78^{d}	80.83^{c}	81.20^{b}	86.13^{a}	0.80

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 a significant difference (P<0.05) in albumin weight, yolk weight and Haugh unit. Horvath and Babinzky (2019) reported a beneficial protective effect of vitamins being evidenced by increase in Haugh unit, albumin and yolk weights. The result of this study indicates the superiority of combining vitamin-C and folate during increased environmental heat load usually at peak periods of the hot season. Also, higher yolk weight in the T4 indicated that a greater proportion of yolk was deposited in the egg of hens consuming vitamin-C as compared to control. This result, similar to (Choi, 2018). This is very useful to the poultry farmer as most commercial buyers of table eggs consider the yolk weight and color especially in the confectionaries subsector. The results of the daily egg production, feed intake records, daily water consumption and body weight gain of heat-stressed layers in hot humid environment treated with vitamin C and folates are presented in Table 4.

Table 4. Daily egg production, feed intake records, daily water consumption and body weight gain of heat-stressed layers in hot humid environment treated with vitamin-C and folates.

Parameters	T1	T2	T3	T4	SEM
Daily Egg Prod.%/Treatment	6.90^{b}	7.56^{ab}	8.15^{a}	8.04^{a}	0.14
Daily Feed intake (kg/bird)	0.19^{b}	0.25^{ab}	0.42^{a}	0.42^{a}	0.04
Feed Conversion Ratio (FCR)	2.02^{a}	1.78^{b}	1.51^{c}	1.51^{c}	0.041
Daily Water Cons.(ltr/bird)	0.36^{a}	0.34^{b}	0.32^{bc}	0.31^{c}	0.03
Initial Body weight (g/bird)	1651.45	1650.14	1654.82	1647.38	2.81
Final Body weight (g/bird)	1670.67^{c}	1672.18^{b}	1679.00^{a}	1672.81^{b}	1.97
Body weight gain (g/bird)	19.22^{c}	22.04^{c}	24.18^{b}	25.43^{a}	1.39

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

The daily egg production per treatment which was (P<0.05) highest in T3(8.15), followed by T4(8.04), T2(7.56) and lowest in T1(6.90); agrees with findings of Ezzat et al., (2019): “vitamin C supplementation in layer birds improved significantly the mean egg production, egg weight and shell thickness”. The daily feed intake values were significantly different (P<0.05) between treated and untreated groups implying a positive impact of single and combined dietary supplementation of ascorbic and folic acids on feed intake of laying chickens exposed to heat stress. The result of this study further explains that the laying chickens in the treated groups (T2, T3 and T4) must have been able to harness the antioxidant effect of vitamin C in neutralization of the free radicals generated during heat stress. The daily water consumption per litre 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 (0.36 ltr/bird), indicating higher penchant for water. This observation is explained as a defensive or survival mechanisms employed by heat-stressed layers that usually elect 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 T2 (0.34 ltr/bird), T3 (0.32 ltr/bird) and T4 (0.31 ltr/bird) strongly suggest that single or combined supplementation with vitamin C and folate will significantly reduce the rush to water by heat-stressed laying birds of hot humid environment. This is because excessive drinking of water will cause wet litter which in turn breed harmful microorganisms in the deep litter poultry pen. T3 and T4 had higher significant values (P<0.05) than T2 and T1. The combined supplementation of vitamin C and other coenzymes such as folate has been shown to increase body weight gain as well as improve growth and performance of birds during heat stress (Nadia et al., 2021). The significantly (P<0.05) lower and similar feed conversion ratio recorded in T3 and T4 (i.e.

folate supplemented treatment groups) show that modern strains of laying hen according to NRC (1994) recommended folate requirement; need more folate because of their improved FCR and productive traits in short period of time.

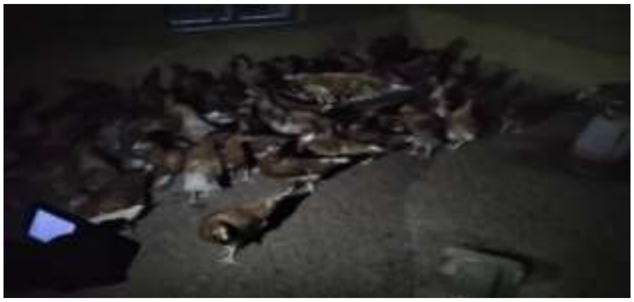

Figure 2. 31 weeks old layer chickens in a deep-litter poultry pen during week 1 and 2 (acclimatization period).

Figure 3. 34 weeks old Isa-Brown Layer chickens in allotted deep-litter poultry pens according to experimental diet specifications.

Figure 4. Experimental layer chickens under observation inside deep-litter pens exposed to increased ambient temperature.

Figure 5. Experimental layer chickens distancing from source of heat inside deep-litter poultry pens (mostly abstaining from feed and water troughs).

CONCLUSIONS

The findings of this study conclude that the interaction of vitamin C and folate significantly reduced heat stress in layer chickens, significantly increased efficient utilization of consumed feed during heat stress and significantly increased daily egg production and egg quality parameters of layer chickens during heat stress. The study concluded that vitamin C supplementation during heat stress significantly improves external and internal egg quality parameters of heat stressed layer chickens. This is necessary for higher economic and nutritional value of eggs laid during increased ambient temperature. It further concluded that combining folate with vitamin C significantly improves digested nutrient utilization during heat stress in layer chickens. This helps the layer chickens to efficiently utilize consumed feed during increased ambient temperature. It finally concluded that combined vitamin C and folate supplementation in layer chickens' diet is required for the production of eggs with significantly lesser damages during transportation and handling.

RECOMMENDATIONS

The findings of this study could help establish technical guidelines for controlling the negative impact of heat stress using vitamin C and folate during peak heat periods of the year. Specifically, It is recommended that layer chickens' diet in the rain forest ecological zone of West African Sub region should be given combined supplementation of vitamin C (at 300 mg/kg feed) and folate (at 1 mg/kg feed).

IMPLICATIONS

The application of this research is that locally-accessible, environmentally-sustainable ameliorative agents like vitamin C and folate minimize the negative impact of increased heat load on egg quality and improve performance in laying hens during the peak hot periods of the year. This will help in the sustainable development of the poultry subsector, particularly in this region. In recent years, there has been an increase in poultry production activities in hot climates, despite the fact that high environmental temperatures negatively affect the growth and production potential of poultry. Today, a great number of the world's poultry species are found in regions where heat load has remained a major management problem at some particular periods of the bird's productive lives and this makes poultry farmers to experience economic loss because ambient temperature conditions intermittently venture outside the zone of thermal comfort for the poultry species.

LIMITATIONS

The study is limited to the parameters studied and could not evaluate the effect of supplementing vitamin C and folate on the biochemistry of heat-stressed layer chickens and egg nutritional qualities.

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