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PANTING BEHAVIOR PATTERNS' EFFECT ON EGG PRODUCTION AND WATER CONSUMPTION OF HEAT-STRESSED LAYER CHICKENS SERVED VITAMIN-C AND FOLATES' SUPPLEMENTED DIET

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Abstract

The ameliorative use of vitamin-C and folates in managing heat-stress-induced panting behavior of layer chickens and its effect on egg production and water consumption were examined. A total of 96 laying hens aged 20 weeks were randomly divided into four treatment groups of 24 birds/group, indicated as T1, T2, T3 and T4. All the treatment groups were subjected to 3-hours supplementary heat for 4-consecutive days of each week for 12 weeks. Formulated diets for birds on T2, T3 and T4 were supplemented with 250mg of vitamin-C, 250mg of Vitamin-C+1mg of folates and 300mg vitamin-C+1mg of folates respectively per Kg of feed. T1 served as the control group without supplementation. The panting rate was significantly highest ($P<0.05$) in T1 while T4 had the least panting rate, suggesting that combining vitamin-C (300mg) and folates (1mg) per kg of feed is an effective ameliorative remedy to control panting in laying hens. T1 consumed the highest volume of water per day. This high penchant for water by T1 explains the defensive or survival mechanism employed by the heat-stressed birds which usually elect to pant, expending large amount of water, which must be replaced if the animal is to maintain effective heat regulation. Daily egg production was significant ($P<0.05$) highest in T3 (8.15) followed by T4 and lowest in T1. These results suggest that the dietary inclusion of vitamin-C (300mg/kg feed) and folates (1mg/kg feed) is a nutritional management strategy to reduce the negative impact of behavioral panting condition on egg production and water consumption.

Keywords: Panting, Heat-stress, Vitamin-C, Folates, Egg

Introduction

Panting behavior remains an indication of chronic heat stress in layer chickens. Panting is a cooling behavioral trait exhibited by many mammals, mostly birds and some reptiles. It is accomplished by the evaporation of water mostly from internal body surfaces such as the tongue, nasal passages, mouth, lungs and air sacs (Campbell, Kenealy & Campbell, 2003).

When exposed to increased ambient temperature, a typical laying chicken will exhibit panting behavior, which increases rate of respiration and results in cooling of the body. Panting behavior exhausts the chicken and expends huge volume of water, which must be replaced if the animal is to maintain effective heat regulation (Campbell et al., 2003). At cellular level, chronic heat stress encourages oxidative damages and oxidative stress. The most obvious is the increased production of free radicals, oxygen species, a decrease in antioxidant defense and it harms cell membranes (Ismail et al., 2004).

Poultry species exhibit panting behavior because they particularly have problems of maintaining homeostasis in warm conditions notably due to their high body temperatures, typically some 3-4^{0}C above mammals, their lack of sweat glands, and the remarkable efficiency of the thermal insulation provided by feathers (Abdi-Hachesoo et al., 2011). As the animals' body temperature rises, its respiration rate increases sharply (Campbell et al., 2003). Under moderate ambient thermal conditions that is less than that of the body; a degree of heat dissipation can occur by physical transfer to the air or ground although a further increase in ambient temperature reduces its efficiency. In such a scenario, panting becomes the direct and efficient means of dissipating body heat by evaporation. According to Richard (1970), for the vaporization of every gram of water evaporated from the respiratory tract represents virtually the same quantity of heat removed from the animal's body. Panting is one of the behavioral responses to overcome increased ambient temperature or heat stress in poultry pens and can be easily measured by observing the birds and recording its behavioural dispositions to increased ambient temperature.

Despite recent considerable research recommending various ways of nutritionally assisting poultry species such as layer chickens overcome the negative impacts of increased ambient temperatures (Nadia et al., 2021, Cayh et al., 2021, Shakeri et al., 2020); many authors have particularly recommended the supplementation of multivitamins and minerals especially in layer chickens (Joubrane et al., 2019, Yakubu et al., 2018, Nadia et al., 2021 and Friday et al., 2021).

Panting in birds has been reported to lead to abnormal condition of increased alkalinity of the blood and tissue, which is associated by loss of CO_2 from the blood (Santin, 2018). This further result in decreasing proportion of ionized calcium in the blood that is available for egg shell formation. It is further worsened by depressed feed intake which results in decreased calcium consumption (David et al., 2012). However, many gaps in knowledge still exist especially in the bird's behavioural responses or behavioural adjustments (particularly in observable behavioral panting dispositions) in prevailing increased ambient temperature and its correlation with egg production and water consumption.

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The welfare of laying chickens remains a subject of interest among livestock breeders, animal welfare advocates and consumers across the globe. Laying chickens' panting behavior is an important indicator of their adaptive welfare to chronic heat stress, induced by increased ambient temperature and provides feedback on how it affects water consumption and egg production. A relationship is believed to exist between the birds' panting behavioral dispositions, water consumption level and quantity of eggs produced within the period of exposure to thermal challenge. This provides the opportunity to achieve specific nutritional supplementation combination targeted at ameliorating the negative impact of oxidative stress and efficient utilization of consumed feed during chronic heat stress.

The general objective of this study is to assess the ameliorative effect of dissolved vitamin-C and folates on observed panting behavior, egg production and water consumption of heat-stressed layer chickens. The specific objectives are:

a. To observe and score behavioral panting dispositions of heat stressed layer chickens served with dissolved vitamin-C and folates' drinking water.
b. To estimate the direct effect of a panting score on daily egg production of heat-stressed layer chickens served with dissolved vitamin-C and folates' drinking water.
c. To estimate the direct effect of a panting score on daily water consumption of heat-stressed layer chickens served with dissolved vitamin-C and folates' drinking water.

The increasing demands for poultry production for efficient nutritional needs of rapidly growing populations of resource-poor tropical warm wet environments and the prevailing global climatic warming, with serious thermal stress on domestic livestock; presents an opportunity for researchers and poultry farmers to find the effect of panting behavioural dispositions of layer chickens under varying degrees of ambient temperatures on daily water consumption and egg production using nutritional ameliorative agents is pertinent.

There is therefore a research need to assess how the panting behavioral dispositions of housed layer chickens impact on the daily egg production and by extension, the daily water consumption of the Isa Brown layer chicken strain. Although a continuous visual monitoring of chicken flock either in battery cages or in deep-litter houses is impractical on commercial scale, a correlation of observed flock responses to chronic heat stress conditions presents an opportunity for targeted ameliorative nutritional management strategy that can reverse the negative impacts of the panting behavior, as well as selection within commercial poultry breeds for heat resilience. The Isa Brown layer chicken is hybrid chicken with sex-linked characteristic brown colour and it thrives in different climates, adapts to different poultry management and housing systems. It is the commonest available commercial chicken layer strain in the warm wet West African Sub region. It is raised for the production of brown table eggs (HENDRIX GENETICS, 2022).

METHODOLOGY

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 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 between 2,500mm to 3,000mm and that of temperature is 22°C to 36°C (N.R.C.R.I., 2021). The experiment lasted for a period of 14 weeks between January and April, 2025.

Experimental 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

The birds were fed layer chickens' ration containing crude protein 16.5%, 2500kcal/kg and supplemented as shown in the 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 250mg of vitamin-C per kg of feed
Treatment 3 (T3)	3-hours duration of additional heat/day and a commercial layers' mash supplemented with 250mg of vitamin-C + 1mg of folates per kg of feed
Treatment 4 (T4)	3-hours duration of additional heat/day and a commercial layers' mash supplemented with 300mg of vitamin-C + 1mg of folates per kg of feed.

Adopted from Okocha and Herbert, (2022)

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.

Experimental animals and management: Isa Brown layer chickens aged 20-weeks (n=96) were purchased from Amobyen Hatchery Nigeria Ltd, Ogun State Nigeria, and used for the study. The laying chickens were managed under deep-litter poultry pens, which were naturally cross-ventilated. The birds were screened and observed for a period of 2-weeks before the commencement of the experiment to prevent external environmental influence. This was to allow the animals acclimatize to their new environment, following which heat treatment 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 medications were strictly observed during the experiment.

Experimental Procedures and Data Collection:

Increment in environmental heat load: A heat-generating solar-powered bulbs were 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 at 34^{o}C for 3-hours during each 4-consecutive days of each week and this heat treatment lasted for 12 weeks during which data were collected.

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 3 consecutive days at the beginning of the experiment and then weekly.

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.

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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.

Panting Observation and scoring: In assessing the panting condition of the experimental birds, visual observations of flock of experimental treatment groups of birds were daily conducted between 9:00am and 3:00pm focusing on panting as described by the panting gradient scores below as described by Okocha (2022). Panting scores were assigned based on the panting dispositions of the experimental birds ranging from variations in the bird's body temperature, postural and behavioural dispositions such as open mouth panting and squatting very close to the ground.

Two trained observers recorded the panting behavior daily using the gradient score descriptions while inter-observer reliability on the assigned panting scores was calculated as described by Cohen (1960) and reported by Islam et al., (2020). The daily visual observations of the panting behavioral dispositions of each treatment group were collated bi-weekly and scored from beginning of week 1 of experimentation to the end of week 12.

Description of panting scores:

0-2.0 NORMAL ≤ 2Hours

a. Usually an open mouth, one-sided or directly squatting close to the floor and panting slowly.

b. Intermittent panting occasionally interrupted by body-scratching, dust bathing or just brief closing of the mouth and drinking of water.

c. Birds may cluster together, or cluster very close to cooler concrete surface.

2.0-3.0 ALERT (From 2 to 3 Hours)

a. Open mouth and progressively steady and slightly heavy panting.

b. A fully standing position and holding the wings out from the sides of the body with tail feathers flapped down.

c. Usually stands close to water source.

d. The panting is rarely interrupted except by introduction of fresh drinking water at room temperature.

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>3.0 DANGER >3 Hours

a. Open mouth severe panting.
b. Fully standing position with wide open droopy wings, tail feathers flapped down.
c. Raised and stretched neck almost like gasping for air.
d. Birds generally stand away from each other in very obvious penguin like position.
e. Birds completely withdraw from feed and water except if fresh drinking water at room temperature is introduced

Table 2: Panting gradient score

Score	Duration
0-2.0 NORMAL (Slow panting)	≤ 2-hours
2.1-3.0 ALERT (Progressively heavy panting)	from 2 – 3hours
>3.0 DANGER (Severely panting)	3-hours and greater

Adopted from Okocha, (2022)

*Panting scores were assigned based on observed average flock panting behavioral dispositions and not on estimation of individual layer chickens' respiration rate.

Statistical Analysis

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

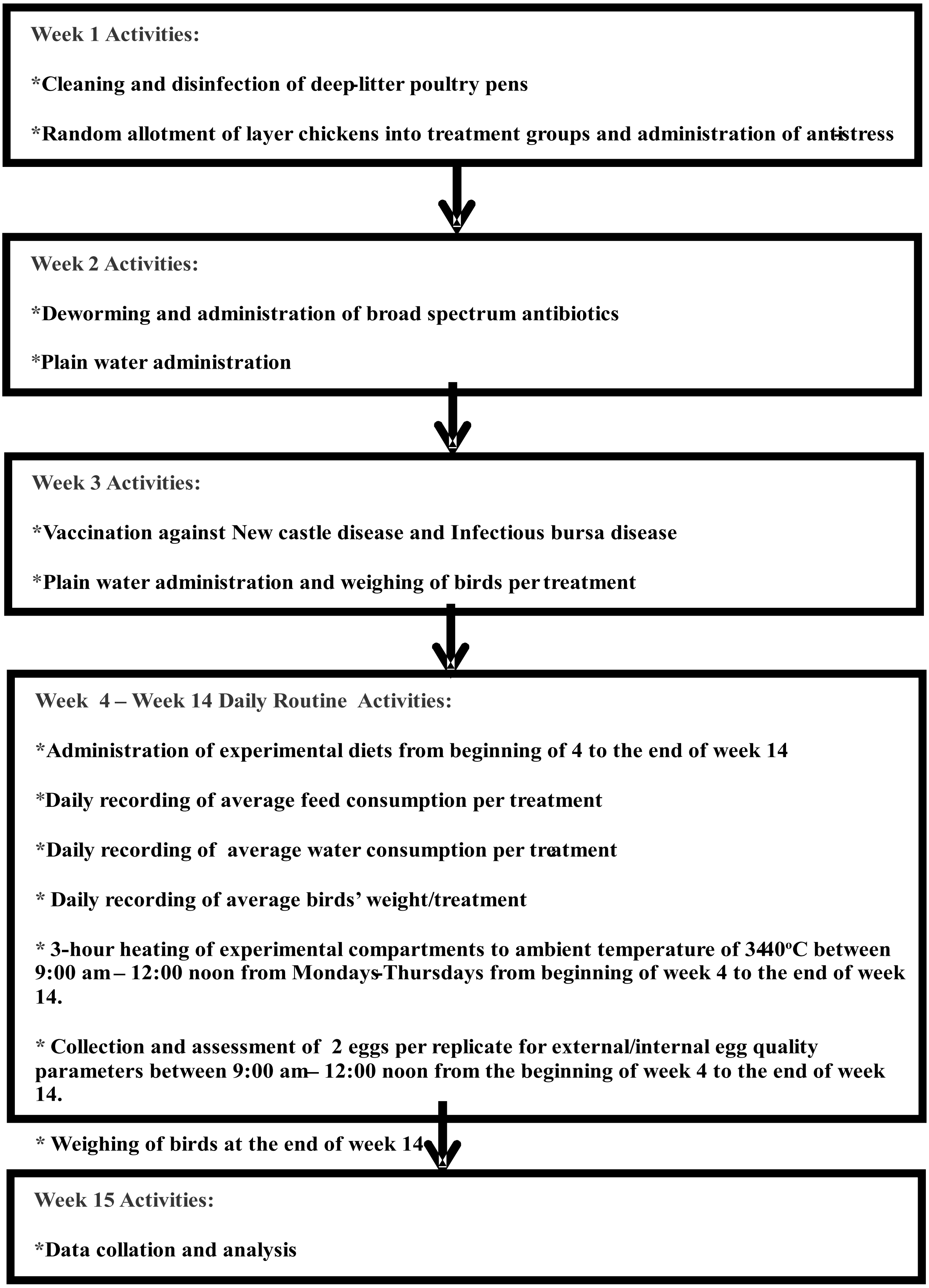

Figure 1: Methodological framework of the experiment
Adopted from Okocha & Herbert, (2022)

FINDINGS

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Panting Observation and Scoring

The data on panting scores of heat stressed layers in hot humid environment supplemented with vitamin C and folates are presented in Table 3.

Table 3: Panting gradient scores of Heat-Stressed Layer Chickens supplemented with Vitamin-C and Folates.

Weeks	T1	T2	T3	T4	SEM
2	1.88^{a}	1.83^{a}	1.79^{a}	1.54^{b}	0.04
4	2.62^{a}	1.93^{c}	2.13^{b}	1.78^{c}	0.08
6	2.44^{a}	2.13^{b}	2.38^{a}	1.91^{c}	0.10
8	3.15^{a}	2.58^{b}	2.82^{ab}	2.04^{c}	0.10
10	2.88^{a}	2.26^{b}	2.44^{ab}	1.79^{c}	0.10
12	3.13^{a}	2.43^{b}	2.49^{b}	1.80^{c}	0.10

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

From the observed panting scores, it is observed that from the beginning of the experimentation to the end of the week 2, the control group T1 (i.e. untreated group), T2 and T3 recorded non-significantly different normal panting scores but are significantly higher than the panting scores of T4. At the 4th week, the treated groups of T2, and T4 still maintained a normal panting score (i.e. slow panting) while T1 and T3 were showing alert in their panting condition i.e. progressively heavy panting.

At the 6th week, T1, T2 and T3 were progressively having heavy panting condition while T4 still maintained a normal panting condition (slow panting). At week 8, all the treated groups of T2, T3 and T4 were progressively having heavy panting condition while T1 had reached a danger score of severe panting condition. At the 10th week, both treated and untreated experimental groups recorded lesser panting scores while T4 slowed down significantly to normal panting score. At the 12th week, T1 had a danger panting score, T2 and T3 were having progressively heavy panting condition while T4 had significantly normal slow panting score.

The significant ($P<0.05$) lower panting scores in experimental treatment groups supplemented with vitamin C indicates that it reduces rate of panting by its antioxidant property of scavenging free radicals (Ismail et al, 2013), where it further reacts with oxygen free radicals to produce hydroascorbyl and transform radicals from lipid stage to aqueous compounds (Ciftci et al., 2005) during chronic heat stress. Horvath & Babinzky (2019) also reported a beneficial protective effect of vitamins during chronic heat stress. The significantly higher ($P<0.05$) panting scores in T1 throughout the experimentation is further explained by

Santin (2018), who stated that at high temperatures, birds pant to enhance evaporative cooling.

However, T4 recorded the lowest significant (P<0.05) panting scores throughout the experimentation which can be explained by the report of Okocha and Herbert (2022) who 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 further establishes the need for higher folates supplementation in Isa Brown layer chickens' diet (1mg/kg of diet) above the National Research Council (1994) recommendation of 0.25mg/kg of diet. This was earlier opined by Satar et al., (2019) who observed that due to the improved production traits in new strains of poultry, a higher folate is required.

Table 4: **Water consumption Heat-Stressed Layer Chickens supplemented with Vitamin-C and Folate.**

Weeks	T1	T2	T3	T4	SEM
2	0.36	0.34	0.32	0.31	0.14
4	0.50	0.35	0.38	0.35	0.16
6	0.46	0.38	0.42	0.38	0.15
8	0.60	0.46	0.50	0.41	0.19
10	0.55	0.40	0.43	0.36	0.17
12	0.59	0.43	0.44	0.36	0.18

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 control group maintained the highest numerical value and had the highest significant difference (P<0.05) throughout the experimentation. This in effect, resulted in the T1 birds consuming more water than other treatment groups. This finding is in agreement with the report of Campbell *et al*., (2003) who stated that panting expends large amount of water, which must be replaced if the animal is to maintain effective heat regulation.

T4 also recorded the least panting rate among the treated groups during the experimentation. This least panting could have influenced the least consumption of water and the higher daily intake of feed. It must have also been as a result of the least rectal temperature observed in T4 in the course of the experiment. This position is strongly supported by Mallonee et al., (1985), Warren et al., (1974) and Baile & Forbes, (1974). This simply suggests that the combined inclusion of ascorbic acid (300mg) and folic acid (1mg) per Kg of feed could be an effective ameliorative remedy to control panting conditions which have deleterious consequences on laying birds especially in hot humid tropical environments where heat stressed remains a challenge to the poultry industry.

Table 4. Daily egg production, feed intake records, daily water consumption and body weight gain of heat-stressed layers supplemented 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 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. 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

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environment. This is because excessive drinking of water will cause wet litter which in turn breeds 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 National Research Council NRC (1994) recommended folate requirement; need more folate because of their improved FCR and productive traits in short period of time.

CONCLUSION AND RECOMMENDATION

From the results obtained in this study, it is concluded that combined supplementation of laying hen's diet with vitamin (at 300mg/kg feed) and folates (at 1mg/kg feed) can alleviate heat-stress induced panting behavioral condition which exerts direct impact on egg production and water consumption. This is evidenced by the ameliorative effect of vitamin-C and folates supplementation on egg production as well as subsequent significant decrease in amount of water consumed during heat stress.

Recommendations

The following recommendations are made:

a. Supplementing poultry diet with vitamin-C and folates should be adopted in the management of climate-induced heat-stress chickens in hot-humid environments.
b. During the peak hot-humid periods, heavy or severe panting (which also signifies climate-induced heat stress) could be prevented by the dietary supplementation with vitamin-C and folates.
c. Combined dietary administration of vitamin-C and folates can be used to improve egg production, minimize excessive water consumption which leads to water feaces, weight gain and production performance of heat-stressed layer chickens.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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