

Original Research Article**Laying Performance, Survival Rate, Egg Quality and Shell Characteristics in Laying Pullets Offered Honey in Drinking Water during Hot Season**

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Abstract

Egg production, survival, egg quality and shell characteristics in laying pullets offered honey in drinking water during hot season were determined using hens (28-week-old, n = 120) allotted to either 0 (CONTROL), 10 (10H) or 20 ml honey/L water (20H) for 16 weeks divided into 4 phases of 4 weeks each. Data on hen-day production (EP), egg weight (EW), length (EL), breadth (EB), shape index (ESI), yolk weight (YW), yolk percentage (YP), albumen height (AH), albumen weight (AW), albumen percentage (AP), Haugh unit (HU), shell weight (SW), shell percentage (SP), shell thickness (ST) and survival (SR) were subjected to ANOVA.

EP was significantly ($P < 0.001$) affected by treatment during phases 1-4. 20H resulted in higher EP than CONTROL in phase 1. In phase 2, EP was similar in CONTROL and 20H, but 10H was lower than the two. Higher EPs were recorded in CONTROL than in honey groups (phases 3, 4). EW was significantly ($P < 0.01$) affected by honey in week 1 only. Birds on 10H laid heavier eggs than CONTROL. 10H hens laid significantly longer ($P < 0.001$) eggs with higher ($P = 0.008$) ESI than control hens while 20H birds had significantly lower values of AH ($P < 0.01$), AP ($P = 0.05$) and HU ($P = 0.05$). Honey had no significant ($P > 0.05$) effect on EB, YW, YP, AW, SW, SP and SR, but improved ($P < 0.05$) ST in the first 2 phases. To ensure improvement in egg production and egg shell thickness in laying pullets during hot season, honey at 20 ml/L water can be offered for 4 weeks.

Keywords: egg; heat stress; phyto-chemical; anti-oxidant; climate change; global warming.

INTRODUCTION

High ambient temperature commonly experienced in poultry houses, in the tropical zones and sometimes during summer in the temperate regions, elicits a series of responses in laying pullets generally termed heat stress (Ayo et al., 2011). Heat stress (HS) causes drastic reduction in egg production, size and quality (Al-Saffar and Rose, 2002), and may be lethal if not controlled (Lara and Rostagno, 2013). Heat-stressed layer birds in a flock respond to the condition by first laying eggs with reduced size, followed by fewer number of eggs laid and later thin-shelled and/or shell-less eggs (Grieve, 2003). Production of thin-shelled eggs results from acid-base perturbations in the blood (respiratory alkalosis) of heat-stressed laying pullets. High blood pH reduces the amount of ionized calcium in the blood. Shell gland in chickens utilizes ionized calcium in secreting egg shell which when in short supply leads to soft eggs. Increasing dietary calcium intake does not correct this problem. This poses a major challenge to poultry production globally.

The onslaught of HS on poultry production is further aggravated as the evidences of climate change with the resultant global warming are becoming more pronounced. Intergovernmental Panel on Climate Change in the Fourth Assessment Report (IPCC, 2007) concluded that besides

other things, the surface air warming in the 21st century by best estimate will range from 1.1 to 2.9 °C for a low scenario and of 2.4 to 6.4 °C for a high scenario (Nardone et al., 2010). Hisas (2011) stated that by 2020, the temperature of the planet would increase by, at least, 2.4 °C above pre-industrial times, going by current business-as-usual path. Climate change is advancing much faster than anticipated. Any procedure that will arrest further increment in global greenhouse gases (GHGs) must be followed.

Most poultry farmers make use of commercial and synthetic anti-stress and anti-oxidants to help chickens cope with HS. Alternatives to the use of chemicals such as anti-stress, anti-oxidants and antibiotics lie in discovery and proper utilization of natural plant materials and extracts that have the properties needed (Bedford, 2000; Wenk, 2003; Vidanarachchi et al., 2005; Ramnath et al., 2008; Zhang et al., 2009; Ali et al., 2010). Various efforts had been geared towards exploration of these materials. One of the promising sources of natural anti-stress/anti-oxidant is honey (Estevinho et al., 2008; Mohamed et al., 2002; Gheldof et al., 2003; Aljadi and Kamaruddin, 2004; Wasagu et al., 2013). In human beings, honey has been used as antibacterial (Adetuyi et al., 2009; Omafuvbe and Akanbi, 2009), antioxidant (Aljadi and Kamaruddin, 2004) and in semen diluents (Oyelowo et al., 2014). It is a thick, viscous and sweet liquid made by bees from the nectar of

flowers, transformed and stored in the honeycombs. Honey is a mixture of many compounds including carboxylic acids, aldehydes, alkynes, nitrites, alkynes and ethers (Adebiyi et al., 2004). Honey is a good example of natural substance that contains phytochemicals such as vitamin C, thiamine, riboflavin, pyridoxine, pantothenic acid, nicotinic acid, phenolic compounds, and enzymes glucose oxidase, catalase, and peroxidase. Its efficacy has recently been reported by Osakwe and Igwe (2015) that honey could be included in layers' feed up to 20% level without any deleterious effect. Honey was said to elicit positive physiological responses in layer chickens under stress conditions and improve egg characteristics.

Presently, information on the use of honey in poultry is scanty and its mode of action still needs to be studied. Therefore, the present study aimed at determining the effect of honey in improvement of egg production and egg internal and external quality characteristics in laying pullets during hot season.

MATERIALS AND METHODS

Experimental location and meteorological observations

This experiment was carried out at Aiyyedoto Farm Settlement, Ojo Lagos, Nigeria (latitude 6° 27' 25"N, longitude 3°12' 21"E and altitude of 36m above sea level). The climate of the experimental site is humid, located in the rain forest vegetation zone of western Nigeria. Wet-and dry-bulb temperatures and relative humidity at the level of the birds in the pen at 08:00 h and 16:00 h were monitored throughout the experimental period. The temperature-humidity index was calculated from relative humidity and wet- and dry-bulb temperature data.

Experimental animals and management

ISA Brown layer chickens ($n = 120$; aged 28 weeks) kept in 3-tier battery cages in an open-sided poultry house were used for the experiment. The birds were apparently healthy at the commencement of the experiment. All recommended vaccinations and medications were adequately carried out. The birds were randomly allocated to three (3) treatments consisting of four replicates and 10 layer birds per replicate. Birds in Groups I, II and III received 0 (CONTROL), 10 (10H) and 20 ml honey (20H) per litre of water for a period of 16 weeks during hot season. The experimental period was divided into 4 phases of 4 weeks each. Weeks 1-4; 5-8; 9-12; and 13-16 represent phases 1; 2; 3; and 4, respectively. Maize-soybean-based standard ration containing 16.5% crude protein, 5% crude fibre, 4% crude fat, 2500 kcal/kg metabolisable energy, 3.5% calcium and 0.45% available phosphorus was given *ad libitum* to the birds and fresh water was offered every morning throughout the experiment.

Data collection

Laying performance: Daily records of number of egg laid by chickens in each replicate were taken and hen-day production (EP) was calculated as the ratio of number of eggs laid daily to number of hens in the pen. Survival rate (SR) was taken as the ratio of number that remained at the end of each week to the number at the beginning of the week in individual replicate expressed in percentage. Measurement of egg weight (EW) was carried out weekly using a sensitive weighing scale to the nearest 0.01 g.

Egg quality: Egg quality assessment was done on all the eggs laid on the third day of every week. Measurement of the longitudinal distance between the narrow and the broad ends was taken as Egg length (EL) with the aid of Vernier caliper of 0.01mm accuracy. Egg breadth (EB) was taken as the diameter of the widest cross-sectional region with the same instrument as above. Egg shape index (ESI) was calculated as the ratio of egg breadth to egg length of individual egg. Measurements of internal qualities of eggs were carried out within 24 h after the eggs had been laid. The egg samples were broken out on a flat, transparent glass plate. Data collected included yolk weight (YW), yolk percentage (YP), albumen height (AH), albumen weight (AW) albumen percentage (AP) and Haugh units (HU).

Shell characteristics: For shell weight (SW), egg shell was air-dried for 72 hours in egg trays. Individual shell weight was determined with an electronic balance with sensitivity of 0.01 g. The ratio of SW to EW expressed in percentage was taken as shell percentage (SP). Shell thickness (ST) was determined by measuring the thickness mean values taken at three spots on the egg (air cell, equator, and sharp end) using a micrometer screw gauge to the nearest 0.01 mm.

Statistical analysis

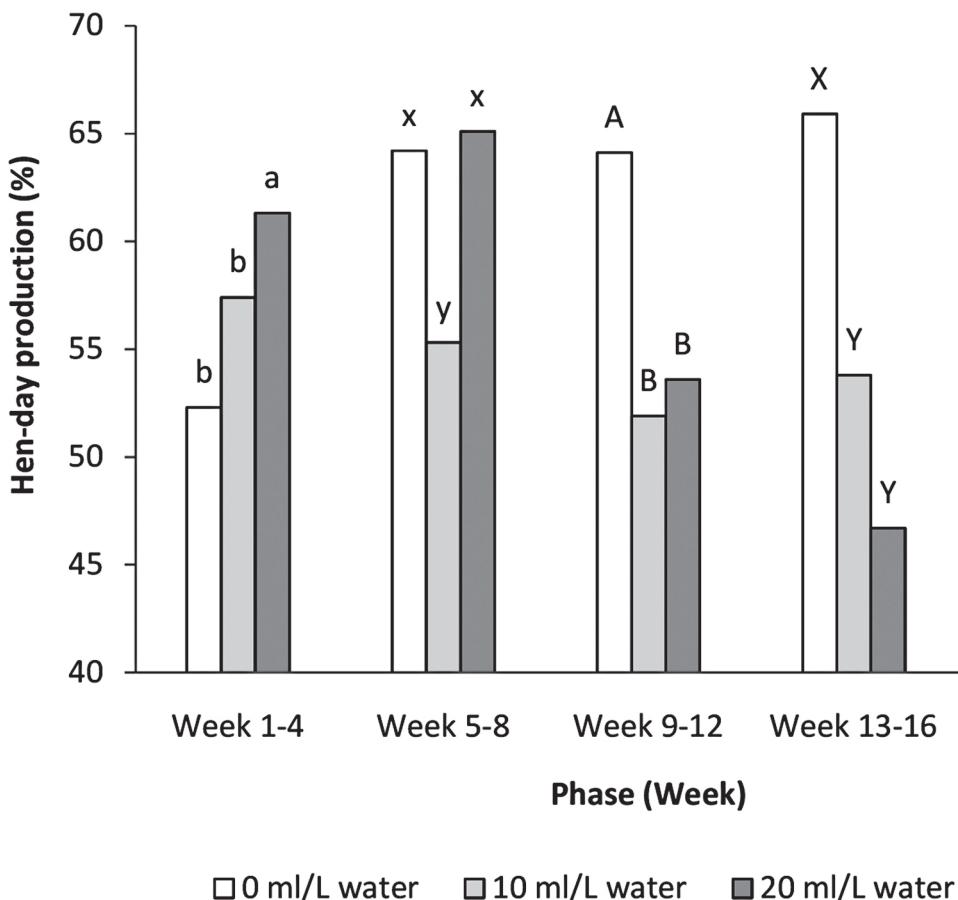
Data collected were subjected to one-way analysis of variance (ANOVA) using SYSTAT (1992) using model: $Y_{ij} = \mu + T_i + \sum_{ij}$; where Y_{ij} = dependent variables, μ = population mean, T_i = i^{th} effect due to addition of honey to drinking water ($i = 1,2,3$), and \sum_{ij} = residual error. Means that were statistically significantly ($P < 0.05$) different were separated with Duncan multiple range test (DMRT).

RESULTS

Table 1 shows the summary of climatic conditions during the experiment. The average dry-bulb and wet-bulb temperatures were 31.5 and 28.0 °C, respectively. Average relative humidity during the experiment was 79.5% while temperature-humidity index of 84.9 was recorded. Egg hen-day production in laying pullets offered varying dosage of honey in drinking water during hot season is presented in Figure 1. Addition of honey in drinking water had significant

Table 1. Summary of climatic conditions during the experiment

	08.00h	16.00h	Average
Dry-bulb temperature (°C)	30.4	32.5	31.5
Wet-bulb temperature (°C)	27.8	28.8	28.0
Relative humidity (%)	82.3	76.7	79.5
THI	83.8	86.0	84.9



^{a,b}Means in week 1-4 with different small letters (a and b) differ significantly ($P < 0.05$)

^{x,y}Means in week 5-8 with different small letters (x and y) differ significantly ($P < 0.05$)

^{A,B}Means in week 9-12 with different capital letters (A and B) differ significantly ($P < 0.05$)

^{X,Y}Means in week 13-16 with different capital letters (X and Y) differ significantly ($P < 0.05$)

^{1,2}Overall means with different numbers (1 and 2) differ significantly ($P < 0.05$)

Figure 1. Egg hen-day production in laying pullets offered varying dosage of honey in drinking water during hot season

($P < 0.01$) effect on EP in Phase 1. Birds that received 20H produced more eggs than birds in CONTROL group (0H). EP in chickens on 10H treatment was not however ($P > 0.05$) different from CONTROL group. In Phase 2, the effect of honey on EP in laying pullets was significant ($P < 0.05$). Chickens on 10H had lower EP than the CONTROL and 20H groups. EP in CONTROL and 20H birds was not different. Phases 3 and 4 show significantly ($P < 0.05$) lower EP in honey (10H and 20H) groups than CONTROL group.

Combining the data for the 16 weeks experimental period, honey groups had significantly ($P < 0.01$) lower EP than CONTROL.

Result of effect of honey in drinking water on egg weight (EW) of laying pullets during hot season is presented in Table 2. Considering EW in phases, there was no significant ($P > 0.05$) difference among the treatment groups. However, significant ($P < 0.05$) effect of honey on EW was recorded only during the first week of the experiment. Birds on 10H

Table 2. Effect of honey on weight of eggs of laying pullets during hot season

Week	Honey (ml/L water)			
	0H	10H	20H	sem
1	56.5 ^b	62.5 ^a	58.8 ^b	1.05
2	58.4	58.2	59.8	1.16
3	58.3	57.6	59.6	1.13
4	56.3	54.4	54.2	1.09
5	56.4	55.9	55.5	0.71
6	54.1	52.7	53.7	1.02
7	56.2	56.1	56.3	0.75
8	53.4	52.9	51.1	1.20
9	56.2	57.9	56.0	1.02
10	59.3	57.5	58.4	1.60
11	61.7	59.6	61.5	1.38
12	60.1	57.9	58.2	1.41
13	58.9	58.8	58.7	1.40
14	56.2	54.4	54.0	1.36
15	56.4	57.3	57.3	1.07
16	58.6	56.3	59.4	1.33
Overall	57.2	56.9	57.0	0.31

^{a,b}Means with different superscripts in the same row differ significantly ($P < 0.05$)

treatment laid heavier eggs than the CONTROL and 20H groups. From week 2 till 16, there were no significant ($P > 0.05$) differences in EW among the groups. Similarly, the difference was not significant in overall EW among the treatment groups.

Table 3 shows the overall effect of honey on egg quality and shell characteristics in laying pullets during 16-week experiment. There were no significant ($P > 0.05$) differences in EB, YW, YP, AW, SW and SP of the 3 honey treatment groups. Hens in CONTROL group (5.59 cm) laid significantly ($P < 0.001$) longer eggs than 10H group (5.52 cm), though not ($P > 0.05$) different from eggs of 20H hens

(5.55 cm). Egg shape index was significantly ($P < 0.01$) affected by honey treatment. Hens on 10H had highest value (78) of the groups, while CONTROL (77) and 20H (76) had similar values. Honey in drinking water had significant effect on AH ($P < 0.01$) and AP ($P < 0.05$) in eggs laid. The values obtained in CONTROL group (0.59 cm and 60.55%) were higher than 20H group (0.54 cm and 56.82%), respectively. Egg shell thickness (Fig. 2) was significantly ($P < 0.05$) affected by honey in drinking water offered laying pullets during hot season. The significant effect was observed in phases 1 and 2. Overall ST showed a significant effect of honey in laying pullets. In the phases 1 and 2, hens on CONTROL treatment laid eggs with thinner shell compared to honey groups, while phases 3 and 4 revealed no difference in ST among the 3 groups. Overall, 20H group had higher ST value than CONTROL but not 10H. Table 4 shows survival rate (SR) in laying pullets as affected by honey in drinking water. The SR for the 3 treatment groups was similar ($P > 0.05$) in all the four phases.

Table 4. Survival rate in laying pullets offered varying dosage of honey during hot season

Phase	Honey (ml/L water)			
	0H	10H	20H	sem
1	97.5	97.5	92.5	4.79
2	87.5	95.0	77.5	6.77
3	80.0	87.5	67.5	9.35
4	75.0	77.5	67.5	10.34

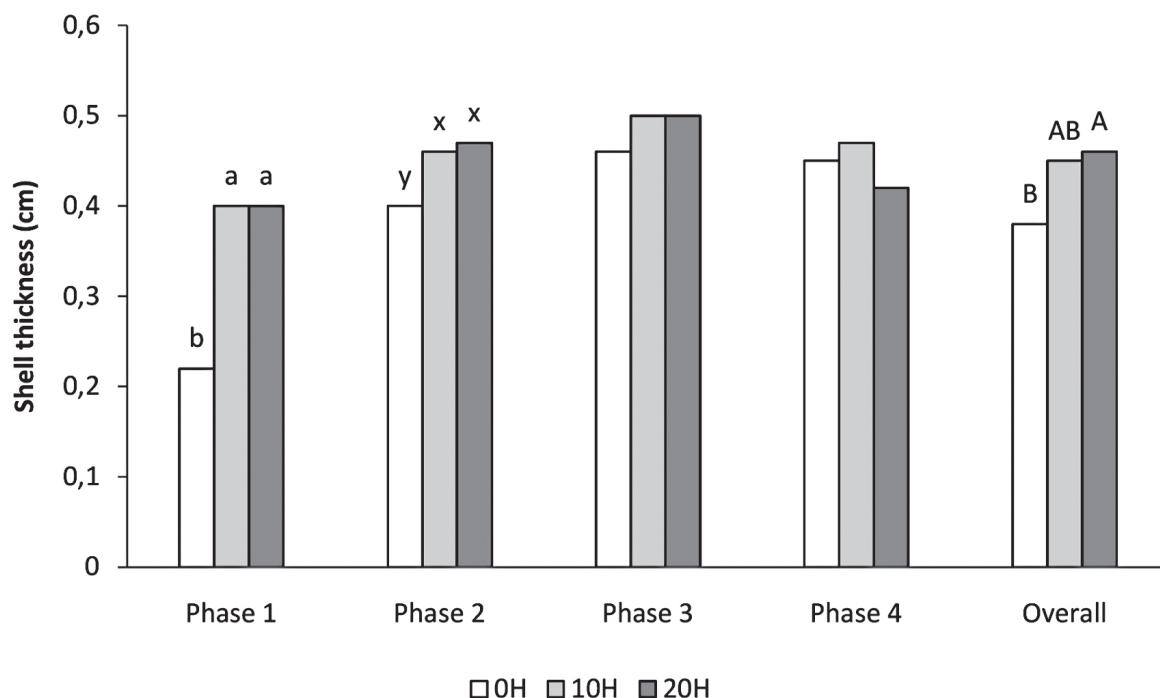
DISCUSSION

Chickens perform optimally under environmental temperature ranging from 16 and 25 °C (Sahin et al., 2006). Various indicators of performance in laying pullets usually nose-dive whenever ambient temperature is higher than

Table 3. Effect of honey on egg quality and shell characteristics in laying pullets during hot season

Parameter	Honey (ml/L water)				
	0H	10H	20H	sem	P
Egg length (cm)	5.59 ^a	5.52 ^b	5.55 ^{ab}	0.013	< 0.001
Egg breadth (cm)	4.32	4.31	4.31	0.008	0.385
Egg shape index (%)	77.0 ^{ab}	78.0 ^a	76.0 ^b	0.01	0.008
Yolk weight (g)	14.95	14.85	13.94	0.202	0.119
Yolk percentage (%)	25.83	25.96	24.86	0.485	0.215
Albumen height (cm)	0.59 ^a	0.57 ^{ab}	0.54 ^b	0.011	0.007
Albumen weight (g)	35.12	34.25	34.14	0.492	0.306
Albumen percentage (%)	60.55 ^a	59.83 ^{ab}	56.82 ^b	1.026	0.026
Haugh unit	76.8 ^a	75.8 ^{ab}	71.7 ^b	1.35	0.019
Shell weight (g)	5.9	6.0	5.7	0.138	0.197
Shell percentage (%)	10.26	10.59	9.99	0.213	0.137

^{a,b}Means with different superscripts in the same row differ significantly ($P < 0.05$)



^{a,b}Means in week 1-4 with different small letters (a and b) differ significantly ($P < 0.05$)

^{x,y}Means in week 5-8 with different small letters (x and y) differ significantly ($P < 0.05$)

^{A,B}Overall means with different numbers (A and B) differ significantly ($P < 0.05$)

Figure 2. Egg shell thickness in laying pullets offered varying dosage of honey in drinking water during hot season

the chickens can cope with (Peguri and Coon, 1991; Al-Saffar and Rose, 2002). Meteorological variables obtained in this study were above thermal comfort zone for egg laying pullets. The average temperature recorded during this experiment was 31.5 °C, about 6.5 °C above the upper critical limit. Attaining the optimal range in the open-sided poultry houses commonly used in the tropics is difficult (Abioja et al., 2012). Average temperature-humidity index during the experiment was such that elicited stress responses from the birds. This accounts for the reduction in egg production over the phases, instead of expected increment. HS causes among other adverse effects reduction in egg production (Mashaly et al., 2004; Deng et al., 2012; Lara and Rostagno, 2013). Reduction in egg production may stem from the behavioural reduction in feed consumption by chickens during hot spell in the bid to reduce metabolic heat production. This causes a low intake of essential metabolites needed for egg formation. Nutrient digestibility is hampered in birds exposed to HS. Another reason for low egg production may be the diversion of nutrients to thermoregulation instead of production activities. Vasodilatation brings much blood and metabolites into the periphery (areas not covered with feathers - skin, comb and wattle) at the expense of the internal organs at the body core.

In the present study, honey was found to be effective in improving egg production in laying pullets only in the first

four weeks of administration while its effect on egg weight was positive only in the first one week. Honey contains various vitamins, electrolytes, natural anti-oxidants and phenolic compounds (Blasa et al., 2006) that may help chickens overcome the negative demands imposed by HS. Comprehensive report on the effect of honey in drinking water of broiler chickens was given by Abioja et al. (2012). Honey reduced pulse and respiratory rate during heat spell and helped improve calcium metabolism in broiler chickens. By-products of honey such as honey slum gum (Babarinde et al., 2011), propolis (Chen et al., 2009) and bee pollen (Wang et al., 2005; Haščík et al., 2012) had been used in broiler production and found to be effective.

Long-term administration of honey to laying pullets was causing a decrease in EP. The reason may be adduced to the higher dosage than needed by the birds or that longer duration of treatment. Recently, Osakwe and Igwe (2015) reported that dietary inclusion of honey up to 20ml improved hen-day production and egg characteristics. This seems to be in contrast to the findings of the present study as some of the parameters are not affected by honey. Analyses of the data were on overall means for 84 days while the present study was phased. This might account for the differences in results obtained. As good as honey is, there is a need for caution in its application in poultry production. Avwioro et al. (2012) reported that there were infiltrations of fat cells in the liver

tissue of albino rats which was dosage-dependent. This may result in non-alcoholic fatty liver disease or in other unpleasant conditions harmful to health if abused. Alagwu et al. (2009) had earlier stated that chronic consumption of unprocessed Nigerian honey resulted in decreased bile flow, increased bile cholesterol and decreased plasma cholesterol in albino rats.

The result obtained on egg length and shape index is similar to the finding of Balnave and Muheeresa (1997) which showed an increase in egg length when layers were given 200mg of Vitamin C/kg diet. The reason for this remains unknown. However, Nikolova and Kocevski (2006) stated that egg shape index is necessary for estimation of egg shell quality. Similarly, an increase in egg shape index was reported by Radwan et al. (2008) in laying hens offered natural antioxidants. But this report disagrees with the results of Tatli (2008) who reported that propolis and honey inclusion in the bird's diet, respectively, did not affect the egg shape index. The present data report that honey does not affect egg breadth, yolk weight, yolk percentage, albumen weight, shell weight and shell percentage does not agree with the findings of Osakwe and Igwe (2015) who stated that 20ml honey per litre water improved egg characteristics and shell quality in laying pullets.

The problem of shell-less and thin-shelled eggs in laying pullets is a major one that is facing the whole world. A trace of likely solution from any corner will always attract attention of poultry farmers and researchers. Egg shell is made up of about 95% calcium carbonate and the remaining 5% shared among phosphorus, magnesium and traces of sodium, potassium, zinc, manganese, iron and copper. Exposure to heat decreases plasma protein and calcium concentration, both of which are required for egg formation (Mashaly et al., 2004). Low quality of egg shell in heat-stressed chickens is not entirely due to dietary calcium deficiency resulting from decreased feed intake, but due to alterations in acid-base balance (Mahmoud et al., 1996; Grieve, 2003). Heat stress results in increased blood pH in chickens exposed to high ambient temperature, a condition known as respiratory alkalosis. The alkalinity of the blood reduces its capability of carrying calcium to the reproductive system for shell formation which may not be corrected by increasing dietary calcium (Emery et al., 1984). Resulting diminished ability of duodenal cells to transport calcium could be a critical factor in egg shell characteristics and skeletal integrity. Respiratory alkalosis restricts availability of bicarbonate for egg shell mineralization and increases organic acid availability which decreases free calcium levels in the blood (Marder and Arad, 1989). Every research effort at ameliorating the detrimental effects of heat stress in laying pullets aims directly or indirectly at reducing this condition.

The present findings show that offering honey to laying pullets did improve shell thickness. This agrees

with Osakwe and Igwe (2015) that gave similar report in laying pullets. This could be linked to improved calcium digestibility and absorption as reported by Tatli (2008) who also reported an increase in egg shell thickness due to certain acid derivates found in propolis. Improvement in shell thickness may be adduced to vitamin C content of honey as vitamin C stimulates 1,25 dihydroxy cholecalciferol and increases calcium mobilization from bone. Plasma calcium is improved by vitamin C supplementation (El-Gendi et al., 1999). Whitehead and Keller (2003) in a review alluded to the fact that vitamin C can be beneficial in countering the adverse effects on shell quality. Honey has been shown to improve tibial weight, density, calcium and phosphorus contents in heat-stressed broiler chickens (Abioja et al., 2012). To ensure improvement in egg production and egg shell thickness in laying pullets during hot season, honey can be offered in drinking water. However, duration of honey administration should not exceed first 4 weeks if the dosage is as high as 20ml/L water.

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