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Natural Thermal Conditioning and Heat Acclimation Improves Heat Tolerance in Layer Chickens During Hot-Dry Season

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Abstract
The aim of this study was to compare the heat tolerance of layer chickens exposed to the hot season during both chick brooding (natural thermal conditioning) and laying (heat acclimation), and that of layers exposed to the hot season only during growth phase. At the beginning of the hot season, 40 layer chickens were randomly selected and classified as Exposed (previously exposed to the hot-dry season during brooding and laying; n=20) and Novel (never exposed to the hot-dry season during brooding and laying; n=20). The results revealed that both morning and afternoon cloacal temperatures were significantly higher ($P<0.05$) at the beginning than peak of the hot-dry in the Novel layers and were also significantly higher in Novel than Exposed layers in the beginning of the hot-dry season. Significantly higher morning respiratory rate was observed in Novel than Exposed group at the peak of the hot-dry season. It was concluded that scheduling the stocking of day-old chicks such that brooding is done at the peak of the tropical hot season and hens have early exposure to the hot season during laying period, could marked enhance thermal conditioning and acclimation to improve heat tolerance and productivity during heat stress.
Keywords: Layer Chickens, Heat stress, day-old chicks, Cloacal temperature, Heat tolerance

Introduction
Chickens are the only source of egg and are the most consumed meat globally with 130.5 million tons of chicken meat produced annually (FAO, 2019). However, the productivity of chickens is limited in hot climates due to their high susceptible to summer heat, as they lack sweat glands to maximise sensible heat loss (Yahav, 2009; Ouchi et al 2021; Habibu et al., 2014). Moreover, an increase in the magnitude and duration of elevated summer temperatures due to adverse effects of global warming has been noted. Thus, increasing the need to identify new adaptation measures against climate change (Sejian et al., 2018; Habibu 2021). Heat stress compromises the thermoregulatory mechanisms and makes the chickens unable to prevent the rise in body temperature above physiological range (Puthpongsiriporn *et al.* 2001; Yahav, 2009; Goel et al., 2021). In laying hens, heat stress reduces feed intake, body weight, egg production and egg quality (Puthpongsiriporn *et al.* 2001; Torki et al., 2018). To combat these negative effects of heat stress, chickens rely on sensible and insensible heat loss, but sensible heat loss becomes the major mean of heat dissipation, when ambient heat load becomes higher than body heat load (Egbuniwe et al., 2015; Habibu *et al*., 2019). At this point, most heat loss occurs through the respiratory route, using evaporative cooling from the vaporisation of moisture from the damp lining of the respiratory tract, including the lungs and air sacs (Tanizawa et al., 2014; Habibu *et al*., 2019).

Previous studies have shown that manipulating some endogenous mechanisms associated with heat dissipation can physiologically improve the tolerance of chickens to subsequent heat-stress exposure, through heat acclimation (Hurwitz et al., 1980; Yahav et al., 1995) or via thermal conditioning (Arjona et al., 1988, Yahav et al., 1997; Yahav and McMurtry, 2001). Thermal conditioning is achieved by exposing chicks at an early age (5 – 6 day-old) to high ambient temperature (up to 40 ^{o}C) to enhance the adaptability of the chickens to heat-stress exposure in later life (Yahav and McMurtry, 2001; Taouis et al., 2002). Thermal conditioning can greatly enhance heat tolerance in later rearing period, when the endogenous heat production has increased, due to onset of metabolic heat generation that is associate with meat/egg production (Tan et al., 2010). Thermal conditioning can reduce metabolic heat production in chickens during heat stress, despite improving protein synthesis and body weight gain (Ouchi et al 2021). Thus, thermal conditioning is a unique phenomenon that accomplishes the establishment of two mutually contradictory production traits: increased productivity and improved heat tolerance (Yahav and McMurtry, 2001).

However, most studies on thermal conditioning in chickens were conducted in broilers and involved the use of artificial heat source (Arjona et al., 1988, Yahav et al., 1997; Yahav and McMurtry, 2001), resulting in a paucity of information on the effect of natural thermal conditioning, mediated by seasonal changes, on thermoregulation and egg quality of layer chickens. If available, results from such studies could guide the farmer to select the most appropriate time to stock and brood chicks, and collectively maximise both heat tolerance and productivity during heat stress (Yahav and McMurtry, 2001; Tanizawa et al., 2014). Similarly, there is paucity of information on the thermoregulatory response of laying chickens to the early changes in ambient variables at the beginning of the hot-dry season. The impacts of these changes may be different from those of the peak of the hot-dry season, due to their novelty and the sudden switch from the unfriendly West African winter (harmattan). Thus, the aim of the current study was to comparatively evaluate the effect of brooding chicks during hot-dry season (natural thermal conditioning) and a previous exposure to the hot-dry season during laying period (natural heat acclimation) on heat tolerance and egg weight of layer chickens. Also, the study compared the impacts of exposure to beginning and peak of the hot-dry season on thermoregulation and egg weight of laying chickens.

Methods, Techniques, Studied Material and Area Description

Study Areas

The study was carried out in a commercial poultry farm (Amir Heritage Farm) located in Kwanan-farakwai, Kaduna State. The pen was a building designed with elevated walls, blocks casting roof system, good ventilation and lighting with automated water system. The chickens were managed in a battery cage system, raised on blocks to permit easy cleaning of the droppings. The waste is piped away and deposited in the soaker-way at the back of the pen, thereafter, to the waste disposal site with a suction machine pump specifically designed for this purpose.

Brooding and feeding

The chicks were house in an environmentally controlled brooding room, maintained at about 39 – 41 ^{o}C for four weeks. The brooding room was heated using charcoal stove and care was taken to ensure the charcoal was not producing smoke, when in the room. Room temperature was monitored hourly using a wet and dry bulb thermometer (Brannan, England). The layer chickens were fed with commercial layer mash (Hybrid Feed Ltd) at 2680 Kcal/kg metabolisable energy. The feed composed of crude protein, fat, crude fibre, calcium, available phosphorus, methionine and lysine at 16.8, 3.6, 4.2, 4.2, 0.5, 0.45 and 0.85 per cent. They were fed three times daily and each chicken consumed an estimated 0.15 kg/day. Clean portable drinking water was given ad-libitum. Standard procedure for brooding in deep litter system, debeaking and vaccination were adequately observed.

Study Design

Two different batches of Isa Brown day-old pullets stocked at six months interval were used for the study. The first and second batches were stocked on 24th April, 2018 (during the hot-dry season) and 10th October, 2018 (after the hot-dry season), respectively. The chickens were moved to a battery cage at 16 week of age. When the first batch of layers were 23 months old and the second batch were 17 months old, a total of 20 chickens were randomly selected from each group and were classified as Exposed (n=20; previously exposed to the hot-dry season during brooding and laying) and Novel (n=20; never exposed to the hot-dry season during brooding and laying). In both groups, parameters were recorded at two different time points in the month of March (beginning-hot season) and April (peak-hot season; Igono and Aliu, 1982). Individual chickens were managed in separate cells of the battery cages in a completely randomised design and each bird was given a tag number.

Determination of Variables

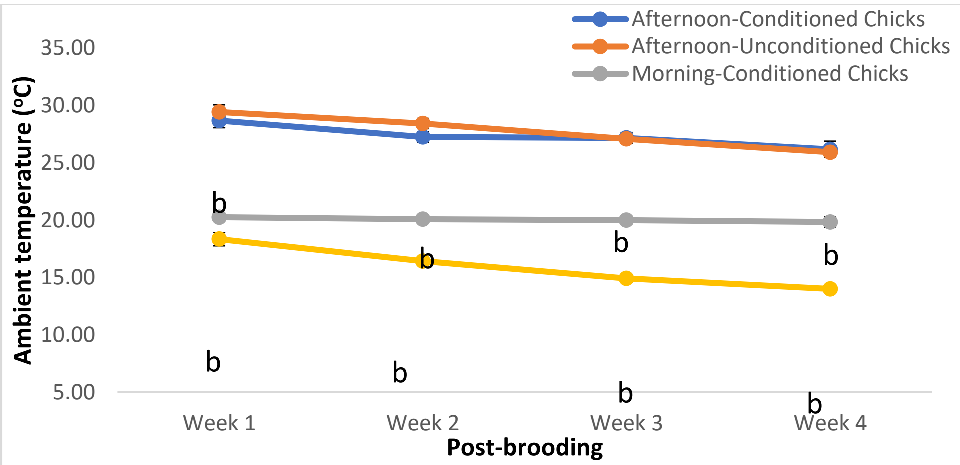
The birds from each group were properly restrained and an infra-red thermometer (place 3 cm from the target site) was used to record the body surface (feather, shank, comb and wattle) temperatures. Feather surface temperature was recorded on the inner surface of the right middle primary feather. The shank surface temperature was recorded on the right lateral surface of the mid-shaft region, while the comb surface temperature was recorded on the right lateral surface of the middle of the comb. Wattle surface temperature was recorded on the right lateral surface of the middle of the wattle. Respiratory rate was recorded by the counting panting breaths of the layers for 60 seconds (Nascimento et al., 2012). Cloacal temperature was recorded using a clinical digital thermometer (Tro-Digitatherm, Hamburg-Germany), inserted approximately 2 cm into the cloaca and in direct contact with the mucosal wall (Habibu *et al*., 2014). Weight of the eggs was taken individually immediately after laying using Metlar MT-5000D Electronic Balance.
Ambient temperatures of the pen were measured using wet and dry thermometer (Brannan, England) in the morning (8:00 h) and afternoon (13:00 h). The relative humidity and temperature humidity index (THI) were respectively derived using Bulletin of the U.S. Weather Bureau No. 1071 and the formula as described by Tao and Xin (2003): THI = 0.85 Tdb + 0.15 Twb. Where: THI = temperature-humidity index, Tdb = dry-bulb temperature and Twb = wet-bulb temperature.

Data analysis
Equality of variances in the data was evaluated using the Bartlett's tests. Values obtained were expressed as mean (± SEM) and subjected to two-way analysis of variance (ANOVA), followed by Tukey's post-hoc test. The statistical package used was GraphPad Prism version 9.0 for windows (2020), San Diego California, USA (www.graphpad.com). Values of $P < 0.05$ were considered significant.

Table 1: Meteorological parameter during the study period period in the early and peak of the hot-dry season.

Parameters	Morning		Afternoon	
	Beginning-Hot season	Peak-Hot Season	Beginning-Hot season	Peak-Hot Season
Ambient Temperature	14.00 ± 0.81 a	16.74 ± 0.51 b	32.93 ± 0.82 c	33.63 ± 0.33 bc
Relative Humidity	29.14 ± 1.31	30.82 ± 3.32	26.571 ± 0.64	28.58 ± 1.95
THI	14.94 ± 0.72 a	17.34 ± 0.43 b	29.49 ± 0.63 c	35.79 ± 0.36 d

A



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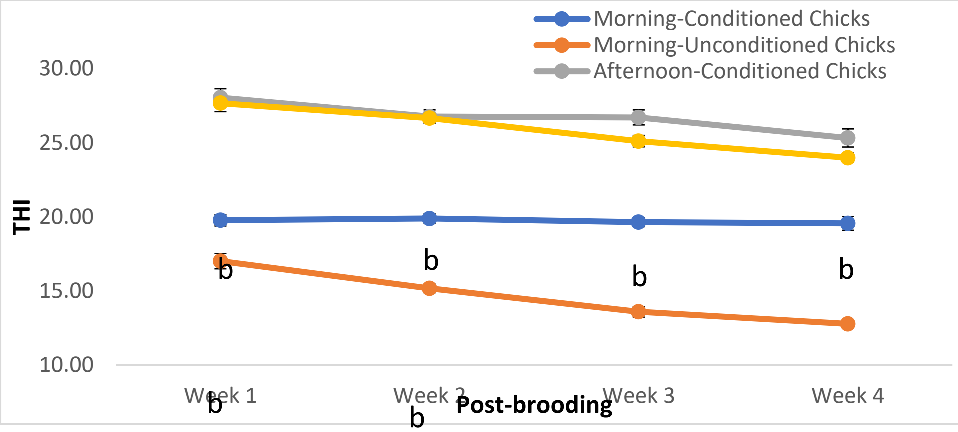

Figure 1: Ambient temperature (A) and THI (B) in the experimental pen after brooding in conditioned and unconditioned chicks. Values with different alphabets indicate significant difference (a, b); THI = Temperature-humidity index.

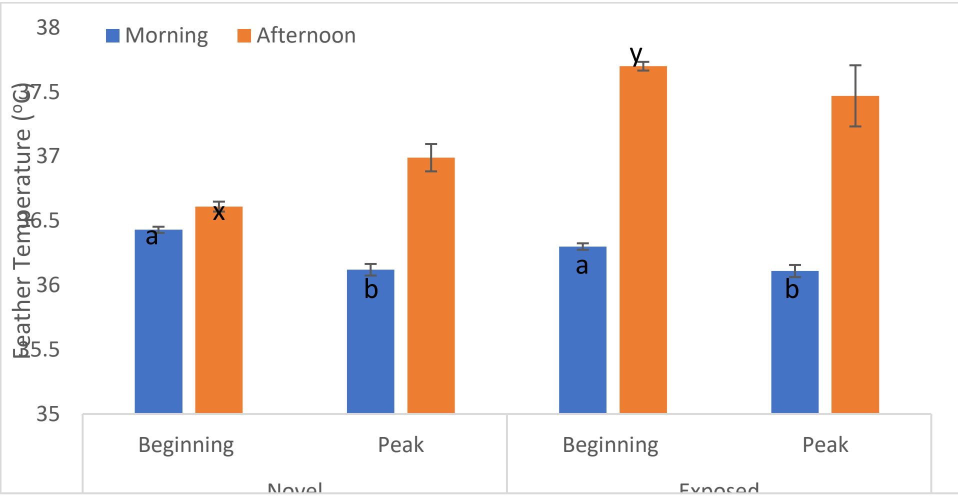

Figure 1: Influence of season and novelty to hot-dry season on feather surface temperature of layer chickens. Values with different alphabets indicate significant difference within (a, b) and between (x, y) exposure type.

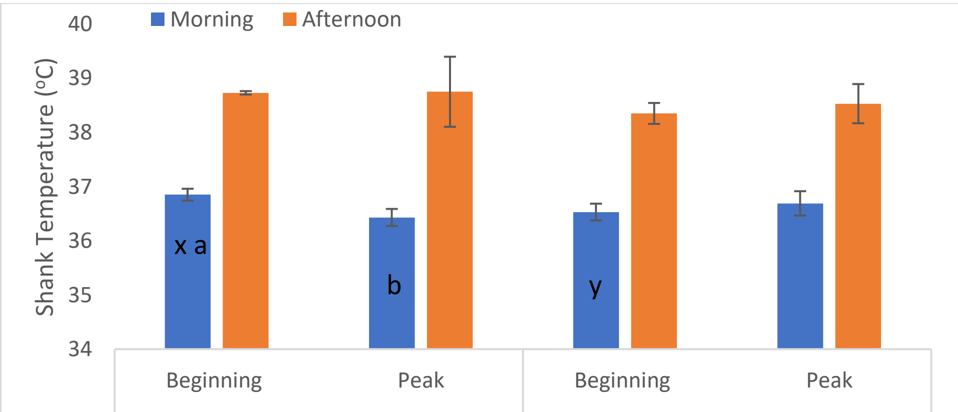

Figure 2: Influence of season and novelty to hot-dry season on shank surface temperature of layer chickens. Values with different alphabets indicate significant difference within (a, b) and between (x, y) exposure type

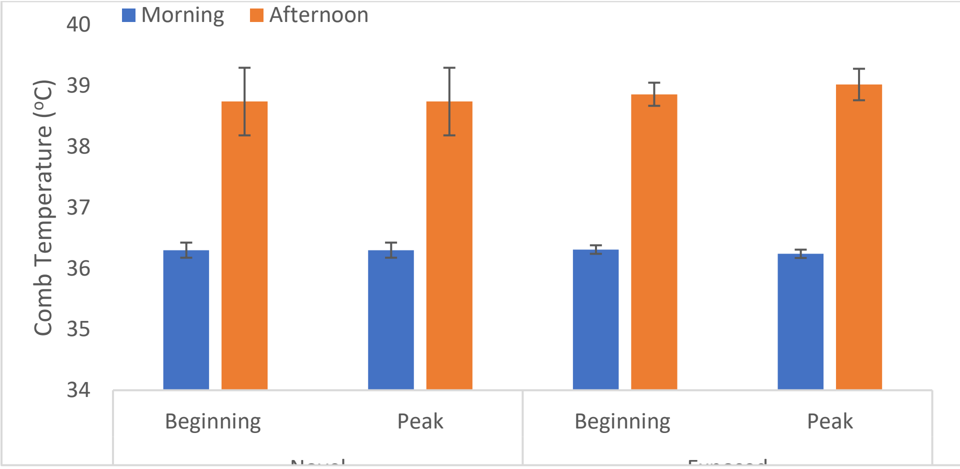

Figure 3: Influence of season and novelty to hot-dry season on comb surface temperature of layer chickens. Values with different alphabets indicate significant difference within (a, b) and between (x, y) exposure type

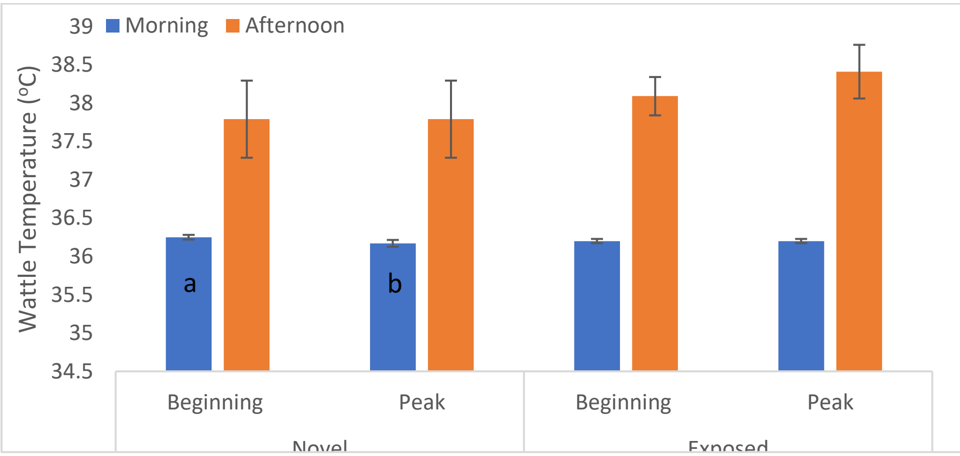

Figure 4: Influence of season and novelty to hot-dry season on wattle surface temperature of layer chickens. Values with different alphabets indicate significant difference within (a, b) and between (x, y) exposure type

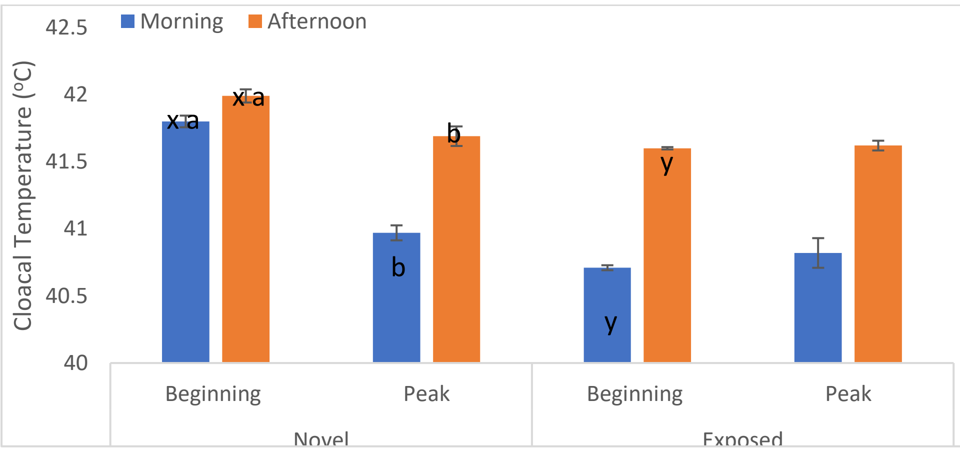

Figure 5: Influence of season and novelty to hot-dry season on cloacal temperature of layer chickens. Values with different alphabets indicate significant difference within (a, b) and between (x, y) exposure type

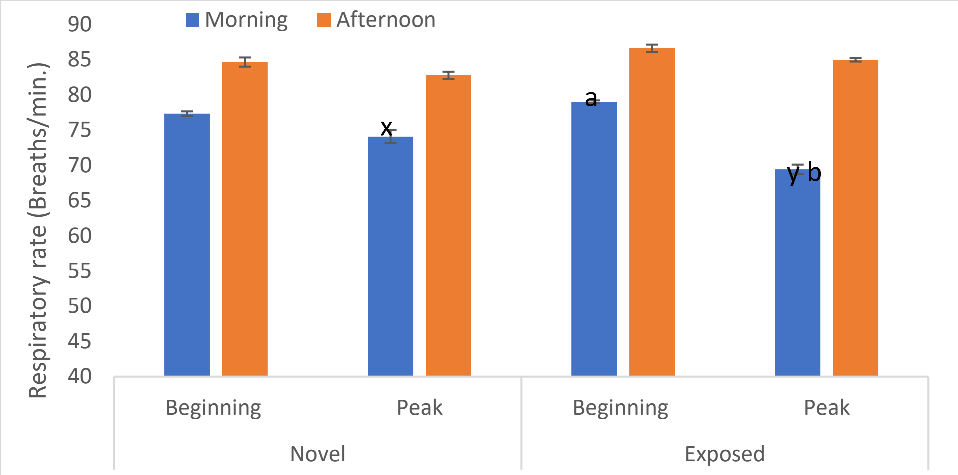

Figure 6: Influence of season and novelty to hot-dry season on respiratory rate of layer chickens. Values with different alphabets indicate significant difference within (a, b) and between (x, y) exposure type

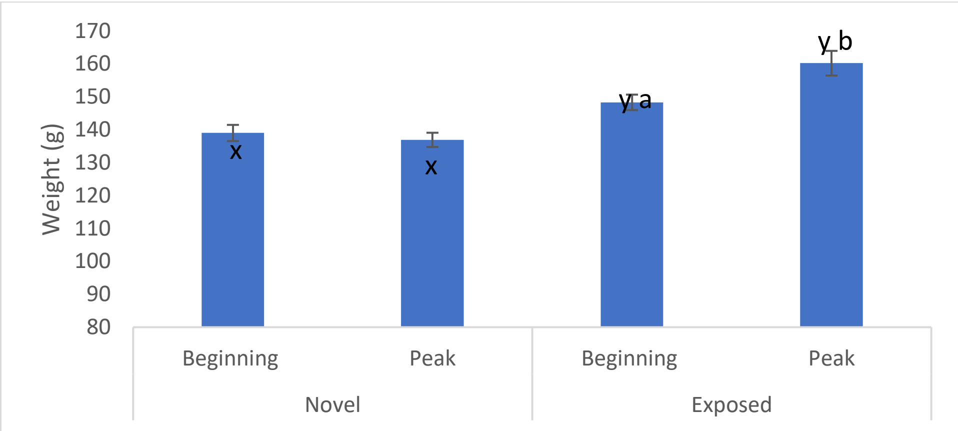

Figure 7: Influence of season and novelty to hot-dry season on egg weight of layer chickens. Values with different alphabets indicate significant difference within (a, b) and between (x, y) exposure type.

Results

Fig. 1 shows the meteorological parameters of the pen within the first four weeks after brooding, while Table 1 shows the meteorological parameters when the physiological variables were recorded during the hot-dry season. In the first four weeks after brooding, the morning ambient temperature and THI of the pen were significantly higher ($P < 0.05$) during the rearing of the Exposed layers than that of the Novel layers. However, during the afternoon hours, no significant difference in ambient temperature or THI was observed during the first four weeks post-brooding between the Exposed and Novel layers. When physiological parameters were recorded in the hot-dry season, values of ambient temperature and THI were significantly higher in the afternoon than morning hours at the beginning and peak of the hot-dry season. The values of THI were significantly higher ($P < 0.05$) in the peak than the beginning of the hot-dry season both in the morning and afternoon hours.

In the layers novel to the hot-dry season, body surface (feather, shank and wattle) were significantly higher ($P < 0.05$) in the beginning than peak of the hot-dry during the morning hours (Fig. 1, 2 and 4). Similarly, in the layers previously exposed to the hot-dry season, feather surface temperature was significantly higher ($P < 0.05$) in the beginning than peak of the hot-dry during the morning hours (Fig.1). Conversely, the feather surface temperature was significantly lower ($P < 0.05$) in novel Exposed layers during the afternoon hours. Irrespective of hour of the day and period of the season, comb surface temperatures show no significant variation (Fig. 3).

In the layers novel to the hot-dry season, cloacal temperatures were significantly higher ($F = 61.6$, $P = 0.001$) in the beginning than peak of the hot-dry both the morning and afternoon hours (Fig. 5). Similarly, cloacal temperatures were significantly higher (F = 61.6, P = 0.001) in novel layers than exposed layers in the beginning of the hot-dry both during the morning and afternoon hours. In the Exposed layers, no significant (P > 0.05) change in cloacal temperature was observed between the beginning and peak of the hot-dry in morning and afternoon hours of the day. Respiratory rate in the Exposed layers was significantly higher (F = 38.7, P = 0.001) in the beginning than peak of the hot-dry in the morning hours (Fig. 6). Similarly, respiratory rate was significantly higher (F = 38.7, P = 0.05) in Novel than Exposed layers in the peak of the hot-dry during the morning hours. At the beginning and peak of the hot-dry, egg weight was significantly lower (F = 29.7, P = 0.05) in Novel than Exposed layers (Fig. 7). Similarly, the Exposed layers had lower (F = 29.7, P = 0.05) egg weight in the beginning than peak of the hot-dry.

Discussion

The recommended thermo-neutral zone for poultry in tropical regions is 12–26 °C (Kingori 2011; Egbuniwe *et al*., 2015), thus in the current study, the afternoon hours posed significant heat stress at the beginning (35.93 ± 0.82 ^{o}C) and peak (36.63 ± 0.334 ^{o}C) of the hot-dry season. Early heat exposure at 3-4 days of age may enhance thermo-tolerance of chickens that would face heat stress in advance (Galal et al., 2015; Ouchi *et al*., 2021). Unfavourable environmental conditions prevailing during the hot–dry season in the Northern Guinea Savannah of Nigeria is well established to induce heat stress, thereby causing a rise in cloacal temperature, reducing egg production and increasing mortality and prevalence of endemic diseases (Ayo et al., 2011; Egbuniwe *et al*., 2015). The ability of chickens to combat such heat challenge is more efficient with repeated thermal conditioning in early life (Ouchi et al., 2021). This might explain the enhanced heat tolerance in Exposed layers as they were exposed to higher ambient temperature in the preceding hot-dry season during their early life, serving as a natural heat-conditioning period. Although, the both Exposed and Novel layers were brooded at 39 – 41 ^{o}C, the Exposed layers are expected to have been exposed to higher ambient temperature from the point of hatching, handling, transportation and off-loading due to the prevailing season, and also higher efficiency of brooding (by maintaining higher brooding temperature) is more likely during hotter seasons. In addition, higher post-brooding temperature, especially in the morning hours in the current study, may have served *as* repeated thermal conditioning to the Exposed layers.

Heat conditioning through early heat exposure on day 3 of age favours a reduction rectal temperature, respiration rate and egg weight in local chickens during adult life (Galal et al., 2015). A reduction in body surface and cloacal temperatures in the peak than beginning of the hot-dry season in the Novel layers, despite the increase in THI, is likely due to acclimation to heat, resulting from continuous heat exposure over a period of one month (Yahav, 2000). However, the lower values of shank surface and cloacal temperatures in the Exposed than Novel layers, infers thermal conditioning, which may have physiologically programmed the layers to adequately activate the mechanism required to combat the effect of heat stress (Tanizawa *et al*., 2014; Ouchi *et al*., 2021). Moreover, the high respiratory rate in the Novel than exposed layers at the peak of the hot-dry season, is likely due to the low heat tolerance of the Novel layers.

Thermal conditioning has been described as a unique management tool that enables high-producing chickens to cope with acute changes in ambient conditions (Yahav, 2000; Tanizawa et al., 2014). In essence, thermal conditioning may be seen to reconcile conflicting challenge faced by the chicken during heat stress, which is, balancing productivity (egg production) and thermotolerance (Yahav and McMurtry, 2001; Tanizawa et al., 2014). Although, acclimation integrates the major physiological processes of improving heat dissipation (Horowitz, 1998) and is a more extensive adaptation than thermal conditioning in chickens, it negatively impacts birds' production capacities (Yahav, 2000; Yahav and McMurtry, 2001). Thus, thermal conditioning appears to be a useful tool for the simultaneous improvement of both thermotolerance and performance (Ouchi *et al*., 2021). On the other hand, heat acclimation has the demerit of markedly reducing body weight, which results from a decline in feed intake and the diversion of body stores to support thermoregulation (Yahav and McMurtry, 2001).

In agreement with the findings of the current study, Galal *et al*. (2015) and Ouchi *et al*. (2021) reported higher cloacal temperature and respiratory rate in chickens that were not conditioned than those that were conditioned to heat stress during subsequent heat exposure. The non-significant change in the cloacal, wattle and shank temperatures in the Exposed layers between the beginning and peak of the hot-dry season, unlike the Novel layers, may be due to enhanced mechanism of heat dissipation, demonstrated by increased respiratory rate and feather surface temperature in the Exposed layer during the morning hours and afternoon hours, respectively. This may help to improve sensible and insensible heat loss in the Exposed layer, due to pre-acquisition or advanced activation of physiological heat tolerance mechanisms. Similarly, Marandure (2007) reported that conditioned chickens more efficiently utilised convection and radiation for heat dissipation during subsequent heat exposure than unconditioned chickens. Furthermore, thermal conditioning has been reported to reduce the magnitude of increase in uncoupling protein gene expression in pectoral muscle and circulating corticosterone level induced by heat challenge and also, reduce circulating T_3 levels during heat exposure (Yahav and Hurwitz, 1996; Uni *et al*., 2001;Taouis *et al*., 2002; Tanizawa *et al*., 2014; Ouchi *et al*., 2021).

In agreement with the current study, Galal *et al*. (2015) reported that thermal conditioned chickens were able to prevent the decrease in egg weight associated with heat stress. This is due to the ability of thermally conditioned chickens to maintain more efficient thermoregulation and thus, invest more energy to sustain production (Taouis *et al*., 2002; Ouchi *et al*., 2021).

Conclusion

Layers chickens that were never exposed to the hot-dry season during their laying period were more susceptible to heat stress and had lower egg weight than the layers previously exposed to the hot-dry season. The beginning of the hot-dry season induced greater thermal stress than the peak of the season (despite the higher THI at the peak of the season), especially in the layer chickens that were never exposed to the hot-dry during their laying period. In the exposed layers, egg weight was higher during the peak as compared to the early hot-dry season. Poultry farmers should schedule the stocking and brooding of their chicks such that the birds will be exposed to the peak of the tropical hot season during brooding to reduce the impact of heat stress on the productivity during laying period. Amelioration against heat stress should commence in the beginning of the hot season, when the impacts of ambient conditions on thermoregulatory compensation and productivity are more grievous.

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