


ORIGINAL RESEARCH

Daily rhythms of cloacal temperature in broiler chickens of different age groups administered with zinc gluconate and probiotic during the hot-dry season

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Keywords

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Abstract

The aim of the experiment was to evaluate effects of zinc gluconate (ZnGlu) and probiotic administration on the daily rhythm of cloacal temperature (t_{cloacal}) in broiler chickens of different age groups during the hot-dry season. One-day-old broiler chicks ($n = 60$) were divided into groups I–IV of 15 chicks per group, and treated for 35 days: Group I (control) was given deionized water; Group II, ZnGlu (50 mg/kg); Group III, probiotic (4.125×10^6 cfu/100 mL), and Group IV, ZnGlu (50 mg/kg) + probiotic (4.125×10^6 cfu/100 mL). Air dry-bulb temperature (t_{db}), relative humidity (RH), and temperature-humidity index (THI) inside the pen, and t_{cloacal} of each broiler chick were obtained bihourly over a 24-h period; on days 21, 28, and 35 of the study. Values of t_{db} ($32.10 \pm 0.49^\circ\text{C}$), RH ($49.94 \pm 1.91\%$), and THI (38.85 ± 0.42) obtained were outside the thermoneutral zone for broiler chickens, and suggested that the birds were subjected to heat stress. Application of the periodic model showed disruption of daily rhythm of t_{cloacal} in broilers on day 21, which was synchronized by probiotic administration. The administration of probiotics or ZnGlu + probiotics to a greater extent decreased the mesor and amplitude, delayed the acrophases of t_{cloacal} in broilers, especially at day 35, as compared to the controls. Overall, the t_{cloacal} values in broiler chickens administered with probiotic alone ($41.25 \pm 0.05^\circ\text{C}$) and ZnGlu + probiotic ($41.52 \pm 0.05^\circ\text{C}$) were lower ($P < 0.001$) than that of the controls ($41.94 \pm 0.06^\circ\text{C}$). In conclusion, probiotic alone synchronized t_{cloacal} of the birds at day 21, and, in addition, decreased t_{cloacal} response most, followed by its coadministration with ZnGlu, the antioxidants may be beneficial in modulating daily rhythmicity of t_{cloacal} and alleviating adverse effects of heat stress on broiler chickens during the hot-dry season.

Introduction

The thermal environmental conditions during the hot-dry season in the Northern Guinea Savannah zone of Nigeria, which prevails from March to May (Dzenda et al. 2011) induce heat stress in pullets (Sinkalu and Ayo 2008), and directly exert adverse effects on the health and welfare of birds (Minka and Ayo 2013; Sinkalu et al. 2015a). Exposure to heat stress affects the circadian rhythms of many physiological variables in livestock, which may disorganize the

circadian system, and consequently the productivity, welfare, and health status of animals (Ayo et al. 1998; Piccione et al. 2013; Minka and Ayo 2016b). The thermoneutral zone for poultry is 18–24°C in the tropics (Dei and Bumbie 2011), but the upper limit of this range is often exceeded in the tropics. In the tropics, the thermoneutral zone for broilers that are 5-week old is 18–24°C; whereas for broilers that are 1-, 2-, 3-, and 4-week old, the thermoneutral zones are 29–33°C, 30–29.5°C, 26–28.5°C, and 23–27°C, respectively (Meltzer 1983; Scheele et al. 2014). High air dry-bulb

temperature (t_{db}) and high relative humidity (RH), characteristic of the hot-humid season result in heat stress (Dei and Bumbie 2011). High t_{db} decreases feed intake, live weight gain, and feed efficiency in broiler chickens (Niu et al. 2009; Azad et al. 2010a), and egg production in laying hens (Franco-Jimenez and Beck 2007; Ajakaiye et al. 2010). It increases cloacal temperature ($t_{cloacal}$) responses (Chowdhury et al. 2012a,b; Egbuniwe et al. 2015) and may cause heat stress in broiler chickens (Soleimani et al. 2010; Singh et al. 2015). After 21 days of age, heat stress causes mortality rate of up to 92.4% (Vale et al. 2010); and high susceptibility, which persists until the broiler chickens attain market age, at days 35–42 (Chepete et al. 2005). Thus, thermal sensitivity of broiler chickens to high t_{db} increases with a rise in body weight (Lin et al. 2004a).

Combating heat stress remains a challenge for the broiler industry in the tropics, which is even aggravated by the changing climatic conditions. The development of novel dietary measures may be beneficial in ameliorating heat stress and enhancing optimum performance in broiler chickens. Harmful effects of different stressors acting on broiler chickens during the hot-dry season may be ameliorated by supplementing the diet of the birds with antistress agents (Erwan et al. 2012), possessing also some antioxidant activity, such as zinc gluconate (ZnGlu) and probiotic (Hasan et al. 2015), which are shown to suppress oxidative stress, and improve the health and growth performance of broiler chickens (Aluwong et al. 2013; Hasan et al. 2015). The effects of ZnGlu alone and its coadministration with probiotics on daily rhythm of $t_{cloacal}$ in broiler chickens, reared during the hot-dry season, have not been investigated. The $t_{cloacal}$ is one of the indices of heat stress, reflecting the core body temperature. It indicates the balance between heat loss and heat gain in broiler chickens (Edgar et al. 2013). Thus, changes in $t_{cloacal}$ during heat stress are used to evaluate the degree of adaptation of broiler chickens to hot-dry conditions (Chen et al. 2013), and level of reactive oxygen species (ROS) in broilers (Azad et al. 2010b), generated in excess during heat stress (Lin et al. 2000).

The aim of this study was to investigate effects of ZnGlu and/or probiotic administration on daily rhythms of $t_{cloacal}$ in broiler chickens of different age groups during the hot-dry season.

Materials and Methods

Experimental site and thermal environmental conditions

The experiment was conducted at the Department of Physiology, Faculty of Veterinary Medicine, Ahmadu Bello University, Zaria ($11^{\circ}10'N$, $07^{\circ}38'E$, altitude 686 m), located in the Northern Guinea Savannah zone

of Nigeria. The broiler chickens after brooding period were kept under natural conditions, without artificial control of the microenvironment. They were, thus, subjected to the naturally-prevailing thermal environmental conditions of high t_{db} and high RH, characteristic of the peak of the hot-dry season in the zone; in April-May, 2015 (Ayo et al. 2011; Dzenda et al. 2011).

Experimental birds, management, and administration of zinc gluconate and probiotic

A total of 60, apparently, healthy broiler chickens (Arbor Acres), comprising both sexes, were used for the experiment. They were kept under an intensive management system in a standard poultry pen, littered with wood shavings. The broiler chickens were given access to water and feeds ad libitum. They were fed with commercial broiler starter (day 0–28) and broiler finisher (day 29–35), produced by Grand Cereals Limited, Jos, Nigeria. The poultry house was made of concrete floor and cement block with aluminum roofing and cardboard ceiling. The dimensions of the pen were 8.4 m \times 5.6 m \times 1.91 m, and the broiler chickens were stocked at 15 birds/m² (Muniz et al. 2006) in order to obtain higher production volumes. The broiler chickens were randomly divided into four groups (I–IV) of 15 birds each: Control (I), ZnGlu (II), probiotic (III), and combination of probiotic + ZnGlu (IV). Both probiotic and ZnGlu were administered daily to the birds individually using a 1 mL-tuberculin syringe for 35 days by the oral route, starting at 1-day old. Each broiler chick was tagged, using a masking tape, on the leg for identification and proper recordings. The study was approved by the Ahmadu Bello University Committee on Animal Welfare and Use, and the management system adhered to the new European Union (EU) council directive 2007/43/EC of laying down minimum rules for the protection of chickens, kept for meat production (European Commission, 2007).

Zinc gluconate 70 mg (PHARMEDIC JSC: Ho Chi Minh City, Vietnam) was dissolved in 50 mL of deionized water and administered at a dosage of 50 mg/kg (NRC, 1994), whereas 1.5 mL/L of the probiotic (*Saccharomyces cerevisiae*) (Montajat Pharmaceuticals, Biosciences Division, Dammam 31491, Saudi Arabia) was administered daily at the concentration of 4.125×10^6 cfu/100 mL, using the competitive exclusion method for 1 week, and according to the manufacturers.

Experimental measurements

Thermal environmental parameters

The t_{dbs} inside the pen were measured by a dry- and wet-bulb thermometer (Brannan^(R), Cumbria, England), and

the RH was calculated using Osmon's hygrometric table (Narinda Scientific Industries, Haryana, India). The t_{db} and RH were recorded every 2 h daily for 3 days; 1 week apart, on days 21, 28, and 35 of the experiment. The thermal environmental parameters were recorded inside the poultry house on each day of the experiment. The temperature-humidity index (THI) for the broiler chickens was determined using the following formula (Tao and Xin 2003):

$$THI = 0.85(t_{db}) + 0.15(t_{wb})$$

where THI = temperature-humidity index for broilers, t_{db} = dry-bulb temperature ($^{\circ}\text{C}$) and t_{wb} = wet-bulb temperature ($^{\circ}\text{C}$).

Measurements of cloacal temperature

The $t_{cloacal}$ values were recorded as an indicator of the core body temperature (Sinkalu et al. 2015b), with the aid of a digital clinical thermometer (KRAUSE digital thermometer^(R), DK-5550, Langeskov, Denmark). The $t_{cloacal}$ was measured, concurrently with the thermal environmental parameters for 3 days only, 1 week apart, in order to reduce the adverse effect of stress due to handling on the birds, known to increase the body temperature (Edgar et al. 2013). On each day of the recording, measurements of $t_{cloacal}$ were taken 12 times using standard procedures (Minka and Ayo 2013) over a 22-h period. After gentle catching and restraining the birds, the $t_{cloacal}$ of each bird was taken by inserting the thermometer about 3-cm deep into the cloaca for 2 min and tilting it to ensure direct contact with the wall of the cloaca. The values of thermal environment and $t_{cloacal}$ were recorded concurrently and bihourly from 07:00 to 05:00 h (GMT + 1) on days 21, 28, and 35 of the study.

Statistical analysis

Data obtained were expressed as mean \pm standard error of the mean (Mean \pm SEM). Cosinor analysis was used to determine the $t_{cloacal}$ daily rhythms of individual birds. The mean mesor (rhythm-adjusted mean), amplitude (half the range of excursion or a measure of the extent of predictable change within a cycle) and acrophase (time of peak) values of the variables of daily rhythm were calculated for each bird and for each time series of the study period. Values were subjected to repeated-measures one-way analysis of variance (ANOVA model-3) and by the cosinor procedure (Refinetti et al. 2007; Piccione et al. 2013), followed by Tukey's multiple comparison post hoc test, using GraphPad Prism 4.0 for windows (GraphPad

Software, San Diego, CA) to compare the differences between the means, obtained from the control and treated broilers. Values of $P < 0.05$ were considered significant.

Results

Variations in thermal environmental parameters on selected days of the study period

The t_{db} on day 28 varied between (29.00–36.00 $^{\circ}\text{C}$) of the study period, and was not different, compared to the value obtained on day 21 (27.00–34.00 $^{\circ}\text{C}$) or 35 (27.00–35.00 $^{\circ}\text{C}$). There was no significant difference in RH and THI values between days 21, 28, and 35 of the study (Fig. 1). From 13:00 h to 15:00 h, t_{db} and THI varied from 31 to 34 $^{\circ}\text{C}$ and 30.30–34.40, respectively (Table 1).

Variations in cloacal temperature of 21-day-old broiler chickens during the hot-dry season

The application of the periodic model showed that the $t_{cloacal}$ of the broilers on day 21 did not exhibit a clear daily rhythm, except for broilers administered with probiotic (Fig. 4). The characteristics of cloacal temperature daily rhythms in broiler chickens of different age groups administered with ZnGlu, probiotic and ZnGlu + probiotic during the hot-dry seasons are shown in Table 2. Although the amplitude of the $t_{cloacal}$ was greater ($P < 0.05$) in control ($2.20 \pm 0.40^{\circ}\text{C}$) as compared to probiotic and ZnGlu + probiotic ($0.7\text{--}1.6^{\circ}\text{C}$) groups, the mesor was lower ($P < 0.05$) in the control group, whereas

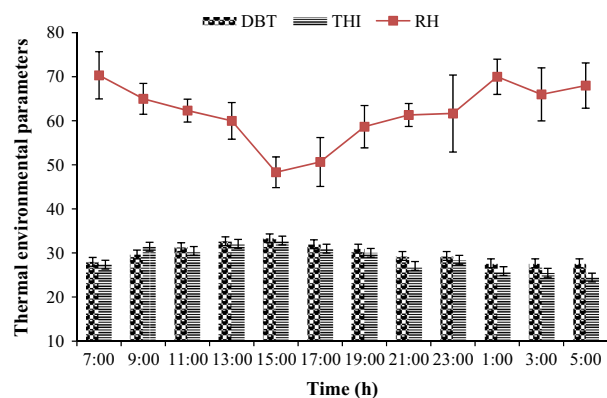


Figure 1. Variations in thermal environmental parameters on selected days of the study period. ($n = 3$). Significant value is at $P < 0.05$. Each data point represents the mean \pm SEM of 60 birds at each period of measurements. DBT, dry-bulb temperature; RH, relative humidity; THI, temperature-humidity index.

Table 1. Variations in thermal environmental parameters on selected days of the study period.

Hours	DBT (°C)	RH (%)	THI
7:00	27–28	60–78	26.60–28.40
9:00	28–31	61–72	29.10–35.10
11:00	30–33	58–67	29.30–32
13:00	31–34	54–68	30.30–34
15:00	32–34	42–54	30.40–34.40
17:00	30–34	42–61	29.10–33.50
19:00	30–32	52–68	29.0–30.80
21:00	28–31	57–66	23.90–30
23:00	28–31	51–79	27.60–29.80
1:00	27–28	66–78	23.80–27.30
3:00	27–29	59–78	23.90–26.60
5:00	27–29	61–78	22.80–26.60
Overall	29.97 ± 0.59	61.86 ± 1.99	28.89 ± 0.81

Values in parenthesis are minimum and maximum. DBT, dry-bulb temperature; RH, relative humidity; THI, temperature-humidity index.

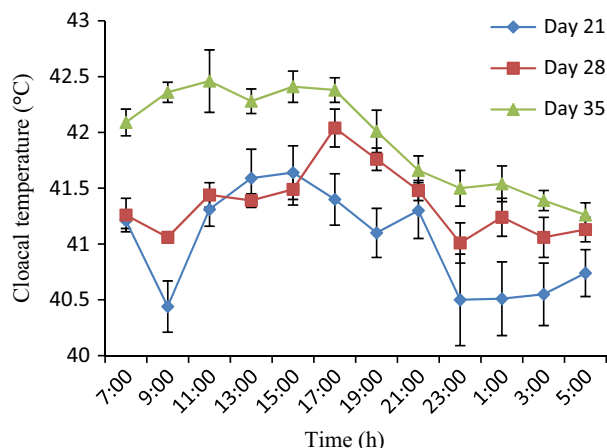


Figure 2. Variations in cloacal temperature of control broiler chickens at days 21, 28, and 35. (*n* = 12). Each data point represents the mean ± SEM of 60 birds at each period of measurements. The CT was higher during the day time at day 21 and decreased during the night time. For each period, measurements were taken at 2-h intervals for a period of 24 h

the acrophases (13:00–15:00 h) were similar in all the groups (Fig. 2).

Variations in cloacal temperature of 28-day-old broiler chickens during the hot-dry season

Figures 2, 3, 4, and 5 show a clear daily rhythmicity of *t*_{cloacal} in all the groups of the broilers on day 28. Rhythm

characteristics of *t*_{cloacal} in control group showed a higher mesor, greater amplitude, and delayed acrophases as compared to probiotic and ZnGlu + probiotic groups (Table 2). Specifically, the *t*_{cloacal} value was higher (*P* < 0.05) in control broiler chickens at 17:00–21:00 h and 1:00 h, but the lowest *t*_{cloacal} value was obtained in probiotic-treated broiler chickens at 11:00 h. ZnGlu-treated group had lower values of *t*_{cloacal} from 19:00 h to 5:00 h (Fig. 3).

Table 2. Characteristics of cloacal temperature daily rhythms in broiler chickens of different age groups administered with ZnGlu, probiotics, and ZnGlu + probiotics during the hot-dry seasons. For each group and on each day measurements were made at 2-h intervals for a period of 24 h.

Groups	Mesor (°C)	Amplitude (°C)	Acrophase (h) (CT°C)
Day 21			
Control	39.90 ± 0.5 ^a	2.20 ± 0.40 ^a	15:00 ± 1.40 ^a (42.10)
ZnGlu	41.15 ± 0.2 ^b	1.15 ± 0.40 ^b	13:00 ± 1.70 ^a (42.30)
Probiotics	41.20 ± 0.2 ^b	0.70 ± 0.03 ^c	14:00 ± 0.90 ^b (41.90)
ZnGlu + probiotics	40.50 ± 0.4 ^a	1.60 ± 0.60 ^d	15:00 ± 1.10 ^a (42.10)
Day 28			
Control	41.35 ± 0.5 ^a	1.25 ± 0.10 ^a	17:00 ± 1.20 ^a (42.40)
ZnGlu	40.75 ± 0.7 ^b	1.15 ± 0.10 ^a	17:00 ± 0.90 ^a (42.10)
Probiotics	40.85 ± 0.3 ^b	0.85 ± 0.04 ^b	15:00 ± 0.90 ^b (41.70)
ZnGlu + probiotics	41.15 ± 0.4 ^b	0.75 ± 0.02 ^b	15:00 ± 1.40 ^b (41.90)
Day 35			
Control	42.20 ± 0.5 ^a	1.55 ± 0.20 ^a	11:00 ± 1.20 ^a (43.60)
ZnGlu	41.70 ± 0.5 ^b	1.30 ± 0.20 ^b	11:00 ± 1.40 ^a (43.00)
Probiotics	41.35 ± 0.4 ^c	1.05 ± 0.10 ^c	13:00 ± 1.40 ^b (42.10)
ZnGlu + probiotics	41.65 ± 0.7 ^b	0.95 ± 0.05 ^c	19:00 ± 1.50 ^c (42.60)

Mean values for each day with different superscript letters along the same row are significantly different at *P* < 0.05; CT, Cloacal temperature.

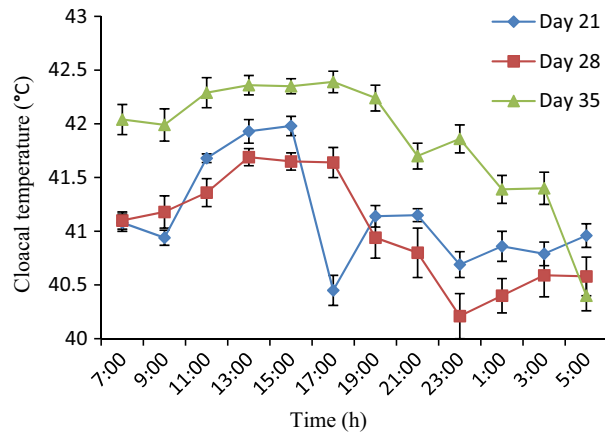


Figure 3. Variations in cloacal temperature of broiler chickens administered with zinc gluconate (ZnGlu) at days 21, 28, and 35. ($n = 12$). Each data point represents the mean \pm SEM of 60 birds at each period of measurements. The CT was higher during the day time at day 35 and decreased during the night time.

Variations in cloacal temperature of 35-day-old broiler chickens during the hot-dry season

On day 35, the broilers in all the groups also exhibited clear daily rhythmicity of t_{cloacal} with a clear ascent during the photophase and a descent during the scotophase (Figs 2, 3, 4, and 5). The mesor of t_{cloacal} in controls was higher ($P < 0.05$) ($42.2 \pm 0.5^\circ\text{C}$), with greater ($P < 0.05$) amplitude ($1.55 \pm 0.02^\circ\text{C}$) and early acrophase ($11:00 \pm 1.20$ h) as compared to other groups that had lower mesor ($41.35\text{--}41.7^\circ\text{C}$), amplitude ($0.95\text{--}1.30^\circ\text{C}$), and delayed acrophases ($13:00\text{--}19:00$ h) (Table 2). The extreme minimum-maximum values obtained in control, ZnGlu, probiotic, and ZnGlu + probiotic groups were as follows: $40.50\text{--}43.60^\circ\text{C}$, $40.40\text{--}43.00^\circ\text{C}$, $40.20\text{--}42.50^\circ\text{C}$, and $40.70\text{--}42.60^\circ\text{C}$, respectively. In general, the probiotic (Fig. 4) and ZnGlu + probiotic (Fig. 5) groups had the lowest t_{cloacal} at each hour of recording both during the day and night.

Discussion

The result showed that the t_{db} values obtained during the study period were predominantly outside the thermoneutral zone of $18\text{--}24^\circ\text{C}$ (Dei and Bumbie 2011) for mature broiler chickens (days 28–42), reared in a hot tropical climate. The t_{cloacal} was measured for 3 days only, 1 week apart, in order to reduce the adverse effects of stress due to handling on the birds, known to increase the body temperature (Edgar et al. 2013). The results of this study showed that thermal environmental conditions of high t_{db} ($27.00\text{--}36.00^\circ\text{C}$) and relatively high RH ($32\text{--}70\%$),

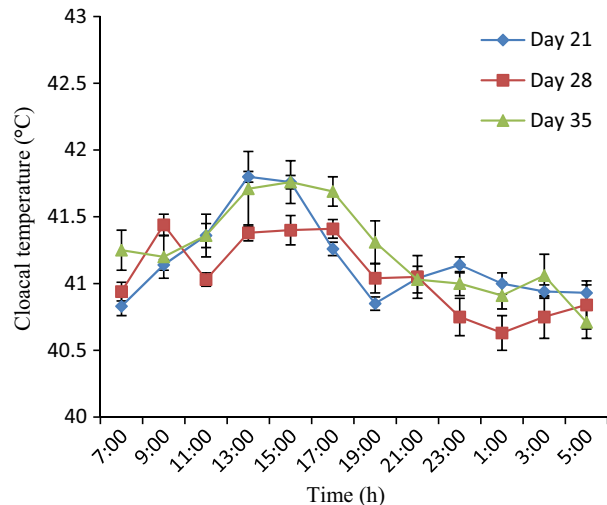


Figure 4. Variations in cloacal temperature of broiler chickens administered with probiotic at days 21, 28, and 35. ($n = 12$). Each data point represents the mean \pm SEM of 60 birds at each period of measurements. Probiotic decreased CT responses, especially at day 28.

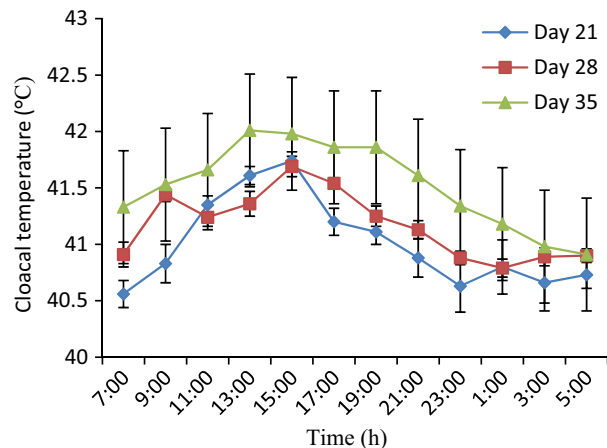


Figure 5. Variation in cloacal temperature of broiler chickens administered with zinc gluconate (ZnGlu) + probiotic at days 21, 28, and 35. ($n = 12$). Each data point represents the mean \pm SEM of 60 birds at each period of measurements. ZnGlu + probiotic modulated CT responses best, especially at day 21.

prevailing during the hot-dry season, were unfavorable for the rearing of broiler chickens, and that they induced heat stress. Elson (1995) reported that the ideal values of t_{cloacal} for broiler chickens vary between 41 and 42°C for a comfortable physiological state, and maximal growth rate and feed intake were observed between the ages of 4 and 8 weeks (Yahav et al. 1995). These values of t_{cloacal} concur with the values of $41\text{--}41.8^\circ\text{C}$ recorded in this study. Robinson et al. (2016) reported that the ideal t_{db}

for broiler chickens within the third week of life is between 26 and 28°C. Similarly, the ideal t_{db} for broilers within the fourth and fifth weeks of life is between 12 and 26°C (Sturkie 1976). Wide fluctuations in RH obtained at days 21 (27–77%), 28 (28–70%), and 35 (26–76%) further aggravated the heat stress during the experimental period. The high THI recorded in this study, fluctuating from 25.55 to 34.50°C, was above the recommended THI value of 20.8 for broilers that are < 21 days' old, indicative of severe heat stress (Purswell et al. 2012; Sinkalu et al. 2015b). The high THI may render evaporative cooling mechanism ineffective in the broiler chickens. The results agree with the findings of Purswell et al. (2012), who reported that as THI exceeds approximately 21°C, the performance of birds significantly declines and their body temperature increases. Since heat stress induces excess production of ROS and consequently, oxidative stress (Lara and Rostagno 2013), the high THI obtained in this study strongly suggests that the birds were subjected to heat stress, especially on day 35 and, consequently, oxidative stress. The finding serves as the rationale to mitigate the adverse effects of heat stress by administration of ZnGlu and/or probiotic, which are potent antioxidants (Zhang et al. 2014; Hasan et al. 2015).

The $t_{cloacal}$ values obtained in the broiler chickens on day 21, except for probiotic group, did not exhibit daily rhythmicity and had the highest range of 4.40°C, but indicates an inability to maintain a stable body temperature of the broiler chickens at this age. The finding was evidence that the thermoregulatory mechanism of the broiler chickens was more stressed in the control birds than any other group. The lowest $t_{cloacal}$ range of 1.4°C recorded in probiotic group showed that probiotic stabilized the $t_{cloacal}$ values in the broiler chickens; thus, maintaining the values at this age at a lower range (40.50–41.90°C) than in any other group. It, therefore, appears that the probiotic exerted thermogenic effect on the broiler chickens and modulated the $t_{cloacal}$ by synchronizing the circadian rhythm of $t_{cloacal}$ at the age of 21 days to normal daily rhythm. Although the acrophase of the $t_{cloacal}$ at day 21 did not differ between the groups, the finding that probiotic group showed the lowest $t_{cloacal}$ value at the acrophase period and had the smallest amplitude as compared to other groups suggests that probiotic, may be used in synchronizing dysfunctional $t_{cloacal}$ rhythms. This finding demonstrates the interrelationship between circadian clocks and metabolism and opens new possibilities for the adoption of nutritional interventions to modulate the circadian clock's function. This requires further investigations. Probiotic facilitates thermogenic activity by increasing the sympathetic activity of the brown and adipose tissues; through enhanced brown

adipose tissue thermogenesis in rats (Tanida et al. 2008), and increases expression of thermogenic proteins (uncoupling protein-2) in mice (Pothuraju et al. 2016). This is a desirable effect. The results of this study showed that probiotic alone, and ZnGlu + probiotic lowered $t_{cloacal}$ values, when compared with the controls. Similarly, Egbuniwe et al. (2015) demonstrated a decrease in $t_{cloacal}$ values in broiler chickens administered with the antioxidants, betaine and ascorbic acid during the hot-dry season. Although the mechanism of action of zinc was not elucidated in this study, it may be linked partly to Zn induction of the ultimate antioxidants, metallothioneins; and protection of protein sulfhydryls. It may also be linked to reduction in OH^- formation from H_2O_2 through the antagonism of redox-active transition metals, such as iron and copper (Powell 2000), and other ROS generated in excess in heat-stressed broilers (Gu et al. 2012; Hao et al. 2012). Probiotic, shown to enhance growth in broiler chickens (Aluwong et al. 2013; Zhang et al. 2014), decreased the $t_{cloacal}$ values; apparently by improving the intestinal microarchitecture in terms of villus height, and crypt depth in heat-stressed broiler chickens (Silva et al. 2010). Further investigations are required at the molecular level to elucidate effects of probiotics on broiler chickens, exposed to heat stress during the hot-dry season.

On day 28, results of $t_{cloacal}$ values showed that ZnGlu and probiotic, either singly or in combination significantly reduced $t_{cloacal}$ values in broiler chickens, especially starting from 17:00 h to 5:00 h, indicating the beneficial effect of the antioxidants in combating the adverse effect of heat stress on broiler chickens during the hot-dry season. The result, unlike on day 21, shows a clear ascent in $t_{cloacal}$ during the photophase and a decent during the scotophase. Furthermore, the results showed that the lowest $t_{cloacal}$ range (1.5–1.7°C) values were obtained in the probiotic and ZnGlu + probiotic groups, indicating that probiotic administration stabilized the $t_{cloacal}$ fluctuations and may be most beneficial in normalizing the $t_{cloacal}$ fluctuations in broiler chickens at the age of 28 days. By decreasing the $t_{cloacal}$ values of the birds, the response of the broiler chickens to administration of antioxidants at this age was beneficial. At this age of 28 days, the thermoregulatory mechanisms of broiler chickens are better developed and their metabolic rate increases rapidly, as evidenced by their rapid growth (Singh et al. 2015). The result of this study showed, for the first time, that the antioxidants, probiotic and ZnGlu have tendency to decreasing the body temperature of the broiler chickens during the last part of photophase period, when the $t_{cloacal}$ values are known to rise, starting from 17:00 h (Fig. 4). This observation was supported by the greater amplitude and delayed acrophase (17:00 h) with $t_{cloacal}$ well above normal values ($42.4 \pm 0.5^\circ\text{C}$) in control

broilers, whereas those of probiotics and ZnGlu + probiotics had smaller amplitude, with t_{cloacal} falling within normal reference values (41.7–41.9°C) at an early acrophase period (15:00 h). The present findings showed that the antioxidants were able to offset the adverse effects of thermal load on the broilers at an earlier stage. The antioxidants are, therefore, beneficial and recommended in modulating and combating adverse effects of exposure of broiler chickens to heat stress, particularly starting from day 28.

At the age of 35 days when the broiler chickens were due for slaughter, the t_{cloacal} of the control group was well above normal reference values of 40–42°C, whereas probiotic and/or ZnGlu administration decreased t_{cloacal} values, starting from 07:00 h to 05:00 h. The finding that probiotic and/or ZnGlu groups had smaller amplitude and delayed acrophases demonstrated that probiotic exerted the most potent effect in reducing the t_{cloacal} values of broiler chickens during the hot-dry season. With increase in age of the broiler chickens, the effect of the antioxidant varied; the lowest reduction in t_{cloacal} values was recorded in broiler chickens treated with ZnGlu at 28 days; but at the age of 35 days, the lowest t_{cloacal} value was obtained in broiler chickens given only probiotic (Fig. 4). The finding showed that age is a crucial factor in the manifestation of responses of broiler chickens to the stressful thermal environmental conditions of the hot-dry season. The t_{cloacal} responses showed that broiler chickens administered with probiotic had the least t_{cloacal} value ($41.25 \pm 0.05^\circ\text{C}$), indicating that probiotic exerted the most potent decrease in body temperature in 35-day-old broiler chickens.

In general, the overall mean t_{cloacal} values recorded in all the groups were within the established normal physiological range (40–42°C) for broiler chickens in the Northern Guinea Savannah zone of Nigeria (Minka and Ayo 2016a). However, at the age of 35 days, especially during the day time, the thermoregulatory mechanism of the birds was adversely challenged, apparently due to increase in metabolic rate and the concomitant effect of heat stress. Thus, the t_{cloacal} values obtained at this period were above the normal reference limits. This finding was evidence that the Arbor Acres breed of chickens at day 35, by inability to maintain homeothermy, has not successfully adapted to the unfavorable thermal environmental conditions in the zone during the day time. Thus at 28- and 35-day-old, it is best to administer ZnGlu and probiotic, respectively to combat the adverse impact of heat stress on broiler chickens during the hot-dry season. Further studies are required to elucidate the effects of ZnGlu and/or probiotic on other body parameters of broiler chickens; involved in the response of the birds to heat stress, especially the hematological, biochemical, and

performance indices. The results of this study, for the first time, demonstrated that ZnGlu and especially probiotic, decreased t_{cloacal} responses in broiler chickens, exposed to heat stress during the hot-dry season; and, in addition, modulated the circadian rhythm of t_{cloacal} in broiler chickens. Consequently, the antioxidants may be beneficial in reducing economic losses, often incurred by farmers due to heat stress in broiler production. Furthermore, the age of the birds is crucial in determining the best antioxidant to be administered; with ZnGlu alone being the best before attainment of 28 days' old; and, thereafter, probiotic alone is the best antioxidant to be administered.

In conclusion, probiotic alone decreased t_{cloacal} response most, followed by its coadministration with ZnGlu; and the antioxidants may be beneficial in alleviating adverse effects of heat stress on broiler chickens during the hot-dry season. For this purpose, ZnGlu and probiotic are best administered at the age of 28 and 35 days, respectively.

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Conflict of Interest

The authors declare no conflict of interest.

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