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Graded levels of Bactofort® modulates tonic immobility and behavioral vigilance responses of broiler chickens during the cold-dry (Harmattan) season

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ABSTRACT

The aim of the study was to determine the effect of graded levels of Bactofort® administration on tonic immobility (TI) and behavioral vigilance responses in broiler chickens during the cold-dry season. Complete randomized sampling was used to assign 300 broiler chicks into five groups. Each treatment was replicated three times, each having 20 birds. Group I served as control and were fed a basal diet without Bactofort® supplementation, group II were fed 0.25 g of Bactofort® per kg of feed, group III were fed 0.35 g of Bactofort® per kg of feed, group IV were fed 0.45 g of Bactofort® per kg of feed, and group V were fed 0.55 g of Bactofort® per kg of feed. Beginning from day-old, broiler chickens in the treatment groups were fed with Bactofort® supplementation daily for 6 weeks. TI was induced by manual restraint and vigilance elicited at self-righting graded for two days, two weeks apart, in 15 labeled broiler chickens from each of the five groups, at 06:00 h, 13:00 h, and 18:00 h, at 28 and 42 days. Each broiler chicken was laid on its back in a U-shaped cradle, covered with cloth. Dry-bulb temperature, relative humidity (RH) and temperature-humidity index inside the broiler chickens' house were recorded, concurrently during the TI and vigilance tests. The mean dry-bulb temperature and temperature-humidity index values fluctuated significantly ($P < 0.0001$ and $P < 0.0001$) as the hour of the day increases, with peak values of 35.53 ± 0.25 °C and 34.52 ± 0.17, recorded at 13:00 h, respectively. The RH fluctuated significantly ($P < 0.0001$) between 42 ± 1.81 % recorded at 13:00 h and 70.17 ± 2.39 % obtained at 6:00 h. There was significant increase in induction trial attempts from group I (control) to group V (0.55 g/kg) at day 28 ($F_{4, 210} = 599.3$; $P < 0.0001$) and 42 [(F 4, 210) = 434.6; $P < 0.0001$] in the treatment groups. The longest duration of TI and behavioral vigilance were recorded at 18:00 h for the control group. The shortest duration of TI and behavioral vigilance was obtained at 18:00 h in group V given 0.55 g/kg of Bactofort®. There was a gradual decrease in the duration of TI ($F_{4, 435} = 12.16$; $P < 0.0001$) and behavioral vigilance ($F_{4, 435} = 56.88$; $P < 0.0001$) in the treatment groups. The results demonstrate that the administration of graded levels of Bactofort® minimized the effect of stressful conditions on the broiler birds, as evidenced by shorter TI duration and decreased behavioral vigilance in the treated groups. In conclusion, the administration of Bactofort® mitigates the adverse effect of heat stress by decreasing TI and behavioral vigilance. The use of Bactofort® supplementation especially at 0.45-0.55 g of Bactofort® per kg of feed may improve the welfare and health of broiler chicken during the cold-dry season.

Introduction

Environmental stress considerably reduces productivity and growth of chickens, causing economic loss for the poultry industry (Azad et al., 2010). The Harmattan is a cold-dry and dust-laden West African north-east trade wind, which blows southward from the

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Sahara desert into the Gulf of Guinea, between the end of November and the middle of March (Anuforom et al., 2007). In northern Nigeria, the Harmattan season is characterized by a wide oscillation of ambient temperature, from low in the night to high in the afternoon, and very low relative humidity (RH). The Harmattan season (November-February) may be thermally more stressful to livestock than either the hot-dry (March-April) or rainy (May-October) season because of its wider range of ambient temperatures and RH (Ayo et al., 2014; Igono et al., 1982).

Probiotics are viable single or mixed cultures of microorganisms that when administered to animals would exert beneficial effects by improving nutrient utilization and enhance the beneficial properties of the gastrointestinal microflora (Lee et al., 2008). Probiotics as beneficial direct-fed bacteria (FDA, 2003) regulate a host's behavior and health through immunomodulation (Goto et al., 2013). Probiotics have been used in poultry, which inhibit pathogenic proliferation and maintain gut integrity, improving growth and performance (Bai et al., 2013; Kabir 2009; Patterson and Burkholder, 2003; Van coillie et al., 2007). Elimination of antibiotics from poultry production has thus encouraged intensive search for alternatives such as probiotics (Park et al., 2016). The use of probiotic in poultry production has been widely accepted and new opportunities arose from the 2006 EU ban on antimicrobial growth promoters (Vilà et al., 2010). Dietary supplementation of probiotic has also been implemented in poultry to counteract the negative effects of heat stress (Lara and Rostagno, 2013). The addition of probiotics, especially Bactofort, to livestock feed were reported to improve the nutritive quality of feed and growth of animals (Martin et al., 1989). Probiotics are increasingly being produced and used for animal nutrition worldwide (Windisch et al., 2008). Several studies have shown that the addition of probiotics (containing *Bacillus subtilis, Lactobacillus acidophilus, Saccharomyces cerevisiae*, and *Enterococcus faecium*) to the diets of broiler chickens ameliorate thermal stress and other stress-induced behaviors (Cramer et al., 2018; Haldar et al., 2011; Jahromi et al., 2016; Khaksefidi and Ghoorchi, 2006; Rahimi and Khaksefidi, 2006; Wang et al., 2018).

Antipredator behaviors such as tonic immobility (TI) and behavioral vigilance in birds are used as indices of fear or stress (Duan et al., 2014; Griesser and Nystrand, 2009; Suzuki et al., 2013; Tisdale and Fernández-Juricic, 2009). TI is an antipredator behavior shown in situations where the animal has been caught by a predator. By feigning dead, there is a better chance to escape in an unguarded moment. Birds confronted with threats exhibit fear-induced freezing called TI, mostly observed in prey species as a defense mechanism (Abe et al., 2013). Most birds frequently scan their environment for potential threats, but these antipredator behaviors usually come at the cost of reduced performance (Watson et al., 2007). Vigilance and TI are important in the evaluation of adaptability of birds to fluctuations or variations in environmental stress factors such as ambient temperature and RH (Forkman et al., 2007). Although TI and behavioral vigilance increases immediate survival of the bird, they could have potential costs such as decreasing growth rate or reproductive output (Zimmer et al., 2011).

The aim of this study was to evaluate the effect of graded levels of Bactofort® on TI and behavioral vigilance of broiler chickens during the cold-dry season.

Materials and methods

Experimental site and location

The experiment was carried out in a poultry house of the National Agricultural Extension Research and liaison Services, Ahmadu Bello University, Zaria (11° 10′ N, 07° 38′ E), Nigeria, located in the Northern Guinea Savannah zone of Nigeria from to November to December, 2017, during the peak of cold-dry season, at an altitude of 706 m above sea level. The width of the broiler chickens' house was 8.7 × 7.5 m, whereas the height was 1.91 m. The broiler chickens' house from the ground to a height of about 0.90 m was made of cement blocks, whereas wire mesh covered from that point to the zinc roof to allow for proper ventilation.

Management of birds

Three hundred birds, belonging to the White Ross breed, were purchased at day-old from a commercial hatchery in Ibadan (06° 56′ N, 03° 43′ E), Nigeria. The birds were vaccinated, via drinking water, against infectious bursal disease (on days 7 and 14) and Newcastle disease (on day 21) using Gumboro and Lasota vaccines, respectively. Each broiler chick was tagged, using a masking tape, on the leg for identification and proper recordings. Deworming was carried out with piperazine solution, dissolved in drinking water at a single oral dose of 160 mg/kg (Aliu, 2007). The broiler chickens were raised on deep litter system and maintained on commercial feeds. The proximate analysis of the feeds given is shown in Table 1. Starter diets were fed to the broiler chicks from day-old to 21 d of life, after which they were placed on finisher diet (day 22-42). The broiler chicks were given access to feed and water ad libitum.

Experimental design

A total of three hundred broiler chicks were divided into five groups by complete randomized sampling. Graded levels of Bactofort® were administered to the treatment groups (groups II, III, IV, and V) via feed from day-old to 42 days. Each group was replicated three times, each having 20 broiler chicks. Broiler chicks belonging to group I served as control and were fed a basal diet without Bactofort® supplementation, group II were fed 0.25 g of Bactofort® per kg of feed, group III were fed 0.35 g of Bactofort® per kg of feed, group IV were fed 0.45 g of Bactofort® per kg of feed, and group V were fed 0.55 g of Bactofort® per kg of feed. Bactofort® is a mixture of probiotic containing the following concentrations of microorganisms: *S. cerevisiae*, 5000 × 10^{12} cells/kg of feed; *B. subtilis*, 2.2 × 10^{12} CFU/kg of feed; *L. acidophilus*, 77 × 10^{12} CFU/kg of feed; and *E. faecium*; 44 × 10^{12} CFU/kg of feed (Biofeed technology inc., Quebec, Canada).

Table 1
Proximate analysis of the broiler diet during the study period

Nutrient contents	Amount in % by weight	
	Pelletized starter feed (1-21d)	Pelletized finisher feed (22-42 d)
Calcium (%)	1.25	1
Available phosphorous (%)	0.45	0.4
Metabolizable energy (kcal/kg)	3308.45	3250
Proximate analysis[a] (%)		
Dry matter	93.72	94.32
Crude protein	20.00	18.36
Crude fiber	4.64	3.47
Oil	5.39	4.83
Ash	11.47	8.91
Nitrogen-free extract	58.60	67.03

[a] Analyzed in the Biochemical Laboratory, Department of Animal Science, Ahmadu Bello University, Zaria, Nigeria.

Meteorological data

The dry- and wet-bulb temperatures were taken at 06:00 h, 13:00 h, and 18:00 h using dry- and wet-bulb thermometer (Brannan (R), Cumbria, England). From the data, RH and temperature-humidity index (THI) at each hour of measurement were calculated. The RH was calculated using Osmon's hygrometric table (Narindra Scientific Industries, Haryana, India). The THI for the broiler chickens was determined using the following formula (Tao and Xin, 2003):

$$THI = 0.85(tdb) + 0.15(twb)$$

where THI = temperature-humidity index for broilers, tdb = dry-bulb temperature (°C), and twb = wet-bulb temperature (°C).

Measurement of tonic immobility responses

Induction of TI was performed on day 28 and 42 as described by Oden et al. (2005) in 15 broiler chickens from each group at 06:00 h, 13:00 h, and 18:00 h by placing a broiler chicken on its back on an improvised cradle. It was then restrained gently with 1 hand placed lightly on its chest for 15 seconds. Each cradle was constructed from Dunlop foams, measuring 50 × 40 × 25 cm and covered with cloth, similar to those used by Saint-Dizier et al. (2008) and Sinkalu et al. (2016). The center of the cradle was scooped to accommodate each broiler chicken on dorsal recumbence. If after releasing the broiler chicken, it righted itself within 2 s, the procedure was repeated. This was done up to 5 times. The resulting TI duration was recorded and if no induction occurred, this was recorded as "0." If the broiler chicken did not resume a standing position after 600 s, the induction process was interrupted (Campo and Carnicer, 1993). A stopwatch was used to record latencies until the bird righted itself (Madec et al., 2008).

Vigilance behavior ranking

The vigilance behavior at self-righting of each TI test observed in the broiler chickens was ranked or scored as described by (Oden et al., 2005) with a slight modification on a scale of 1 to 3, with 1 representing fearlessness and 3 fearfulness. Briefly, vigilance behavior of each broiler chicken at self-righting was ranked as follows: 1, if the process of self-righting was quick and spontaneous; 2, if the process was slow and with a short vigilance period (peeping and vocalization); and 3, if the process was very slow and with a prolonged period of vigilance.

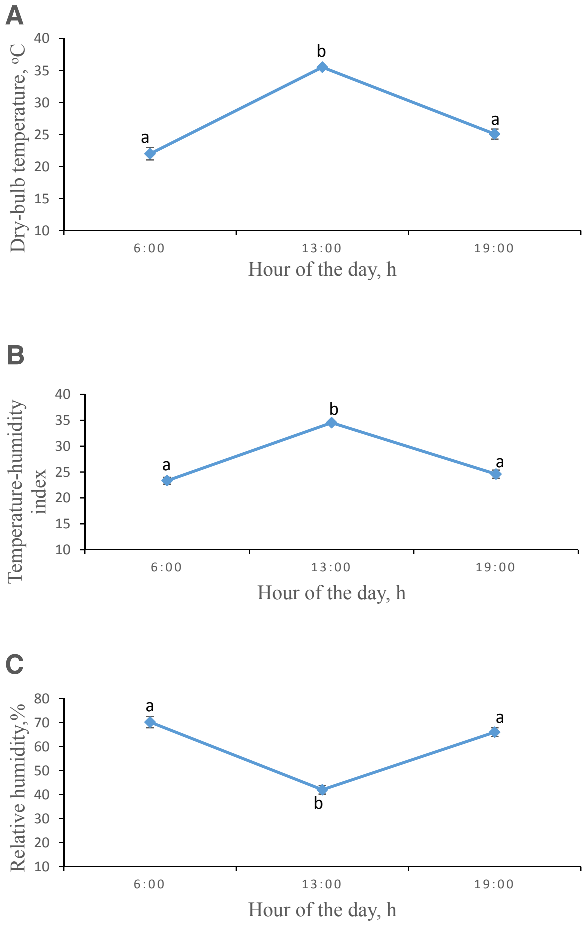

Figure 1. Daily fluctuations in dry-bulb temperature (A), temperature-humidity index (B), and relative humidity (C) inside the broiler pen during the study period (mean ± SEM). [a,b] Means having different superscript letters are significantly ($P < 0.05$) different.

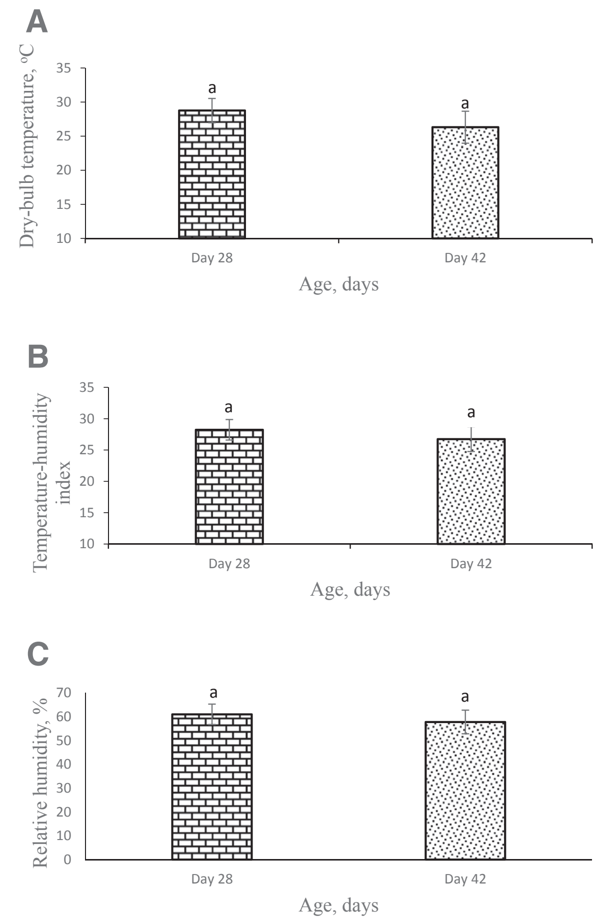

Figure 2. Thermal environmental data inside the broiler pen at days 28 and 42 during the study period (mean ± SEM). [a,b] Means with different superscript letters are significantly ($P < 0.05$) different. A = Dry-bulb temperature, B = Temperature-humidity index, C = Relative humidity.

Table 2
Effects of graded levels of Bactofort® on the number of induction attempts in broiler chickens during the cold-dry season (n = 15)

Age, days	Hour, h	Groups					Hour of the day		Treatment		Interaction	
		Group I	Group II	Group III	Group IV	Group V	*P* values	*F* values	*P* Values	*F* values	*P* values	*F* values
28	06:00	1.00 ± 0.00^{a,1}	1.12 ± 0.02^{a,1}	1.39 ± 0.03^{b,1}	1.64 ± 0.05^{c,1}	1.93 ± 0.04^{d,1}	<0.0001	126.8	<0.0001	599.3	<0.0001	30.74
	13:00	1.01 ± 0.01^{a,1}	1.16 ± 0.02^{a,1}	1.46 ± 0.02^{b,1}	1.99 ± 0.04^{c,2}	2.60 ± 0.08^{d,2}						
	19:00	1.00 ± 0.00^{a,1}	1.01 ± 0.01^{a,2}	1.30 ± 0.02^{b,2}	1.57 ± 0.03^{c,1}	1.76 ± 0.03^{d,1}						
	Mean ± SEM	0.98 ± 0.02^{a,1}	1.10 ± 0.01^{a,1}	1.38 ± 0.02^{b,1}	1.49 ± 0.03^{c,1}	2.10 ± 0.06^{d,1}						
42	06:00	1.06 ± 0.04^{a,1}	1.18 ± 0.02^{a,1}	1.48 ± 0.02^{b,1}	1.78 ± 0.03^{c,1}	1.94 ± 0.03^{d,1}	< 0.0001	52.48	< 0.0001	434.6	<0.0001	11.43
	13:00	1.01 ± 0.01^{a,1}	1.14 ± 0.03^{a,1}	1.45 ± 0.05^{b,1}	1.80 ± 0.04^{c,1}	2.32 ± 0.08^{d,2}						
	19:00	1.00 ± 0.00^{a,1}	1.01 ± 0.01^{a,2}	1.30 ± 0.02^{b,2}	1.56 ± 0.03^{c,2}	1.76 ± 0.03^{d,3}						
	Mean ± SEM	1.00 ± 0.03^{a,1}	1.11 ± 0.02^{a,1}	1.42 ± 0.02^{b,2}	1.73 ± 0.02^{c,2}	2.03 ± 0.04^{d,1}						

Age		Treatment		Interaction	
$P < 0.027$	$F = 4.825$	$P < 0.0001$	$F = 402.7$	$P < 0.0001$	$F = 7.261$

Values are expressed as mean ± SEM.
Group 1 = control, group II = 0.25 g/kg, group III = 0.35 g/kg, group IV = 0.45 g/kg, and group V = 0.55 g/kg.
[a,b,c,d]Means for the same row having different superscript numbers are significantly ($P < 0.05$) different.
[1,2,3]Means for the same column having different superscript letters are significantly ($P < 0.05$) different.

Statistical analysis

Data obtained were expressed as mean ± standard error of the mean (mean ± SEM). Before analyses, raw data were subjected to normality of distribution using Kolmogorov-Smirnov test. The data were found to be normally distributed. A multiway analysis of variance was used to determine the effect of treatments (graded doses of Bactofort®), age, and hour of the day on the broiler chicken. The multiple means were compared using Tukey (1949) multiple range test. Pearson's correlation analysis was used to determine the relationship between TI induction trial attempts and dry-bulb temperature (DBT), THI, and RH. The analysis of variance and Spearman's correlation statistical analysis were carried out using GraphPad Prism, version 6.01, for Windows (GraphPad software, San Diego, CA, USA; www.graphpad.com). *P* values of < 0.05 were considered significant (Snedecor and Cochran, 1994).

Results

Thermal environmental parameters inside the broiler pen during the study period

The thermal environmental parameters obtained during the study period are shown in Figures 1 and 2. The minimum and maximum DBT values during the study period were 19.0°C and 36.5°C, respectively. The mean DBT (35.53 ± 0.25 °C) fluctuated significantly ($P < 0.0001$) as the hour of the day increases, with peak values recorded at 13:00 h. The minimum, maximum, and mean RH values recorded during the study period were 38 %, 76 % and 59.38 ± 3.20 % with peak value at 06:00 h (Figure 1). There was no significant difference ($P > 0.05$) between the DBT, THI, and RH recorded at day 28 and 42 (Figure 2).

Effect of age and hour of the day on tonic immobility induction attempts

The highest numbers of induction trial attempts were 2.60 ± 0.08 and 2.32 ± 0.08 obtained in group V (0.55 g/kg) at 28 and 42 days (they were recorded at 13:00 h), respectively. There was significant increase in induction trial attempts from group I (control) to group V (0.55 g/kg) at day 28 ($F_{4,\ 210} = 599.3$; $P < 0.0001$) and 42 [(F 4, 210) = 434.6; $P < 0.0001$] in the treatment groups (Table 2).

Effect of hour of the day on tonic immobility duration and behavioral vigilance

The longest duration of TI (165.97 ± 28.79 s) and behavioral vigilance (2.37 ± 0.12) was recorded at 18:00 h and 13:00 h for the control group, respectively (Tables 3 and 4). The shortest duration of TI (69.87 ± 12.11 s) and behavioral vigilance (1.10 ± 0.06) was obtained at 18:00 h in group V given 0.55 g/kg of Bactofort®. The duration of TI and behavioral vigilance fluctuated from 6:00 h to 18:00 h in the Bactofort®-treated groups. The duration of TI and behavioral vigilance decreases gradually as the dosage of Bactofort increases from 0.25 to 0.55 g/kg. There was a gradual decrease in the duration of TI ($F_{4,\ 435} = 12.16$; $P < 0.0001$) and behavioral vigilance ($F_{4,\ 435} = 56.88$; $P < 0.0001$) in the treatment groups as the doses of Bactofort® is increased from 0.25 g/kg in group II to 0.55 g/kg in group V (Tables 3 and 4).

Table 3
Effects of hour of the day on tonic immobility duration (seconds) in broiler administered graded level of Bactofort® during the cold-dry season (n = 15)

Hour	Group I (0)	Group II (0.25 g/kg)	Group III (0.35 g/kg)	Group IV (0.45 g/kg)	Group V (0.55 g/kg)	Hour of the day		Treatment		Interaction	
						P values	*F* values	*P* values	*F* values	*P* values	*F* values
06:00	158.03 ± 10.71^{a,1}	151.50 ± 20.58^{a,1}	120.50 ± 16.32^{b,1}	100.87 ± 13.35^{c,1}	87.63 ± 13.16^{d,1}	0.0481	6.864	< 0.0001	12.16	0.855	0.5018
13:00	157.50 ± 22.93^{a,1}	119.47 ± 13.98^{b,2}	95.90 ± 8.65^{c,2}	90.93 ± 11.94^{c,1}	82.70 ± 9.15^{d,1}						
18:00	165.97 ± 28.79^{a,1}	110.60 ± 17.67^{b,2}	80.03 ± 9.59^{c,2}	77.40 ± 9.89^{d,2}	69.87 ± 12.11^{e,2}						

Values are expressed as mean ± SEM.
Group 1 = control, group II = 0.25 g/kg, group III = 0.35 g/kg, group IV = 0.45 g/kg, and group V = 0.55 g/kg.
[a,b,c,d] Means for the same row having different superscript numbers are significantly ($P < 0.05$) different.
[1,2] Means for the same column having different superscript letters are significantly ($P < 0.05$) different.

Table 4
Effects of hour of the day on behavioral vigilance in broiler administered graded level of Bactofort® during the cold-dry season (n = 15)

Hour	Group I (0)	Group II (0.25 g/kg)	Group III (0.35 g/kg)	Group IV (0.45 g/kg)	Group V (0.55 g/kg)	Hour of the day		Treatment		Interaction	
						P values	*F* values	*P* values	*F* values	*P* values	*F* values
06:00	$2.34 \pm 0.09^{a,1}$	$1.80 \pm 0.09^{b,1}$	$1.60 \pm 0.10^{c,1}$	$1.57 \pm 0.09^{c,1}$	$1.33 \pm 0.09^{d,1}$	0.0006	7.583	< 0.0001	56.88	0.863	0.4906
13:00	$2.37 \pm 0.12^{a,1}$	$1.70 \pm 0.10^{b,1}$	$1.53 \pm 0.10^{c,2}$	$1.40 \pm 0.09^{d,2}$	$1.30 \pm 0.10^{e,1}$						
18:00	$2.33 \pm 0.13^{a,1}$	$1.47 \pm 0.10^{b,2}$	$1.33 \pm 0.11^{c,3}$	$1.23 \pm 0.08^{d,3}$	$1.10 \pm 0.06^{e,2}$						

Values are expressed as mean ± SEM.
Group 1 = control, group II = 0.25 g/kg, group III = 0.35 g/kg, group IV = 0.45 g/kg, and group V = 0.55 g/kg.
[a,b,c,d,e] Means for the same row having different superscript numbers are significantly ($P < 0.05$) different.
[1,2] Means for the same column having different superscript letters are significantly ($P < 0.05$) different.

Effect of age on tonic immobility duration and behavioral vigilance

The duration of TI decreases in the Bactofort-treated groups from day 28 to 42. The duration of TI was significantly lower in 42-day-old broilers when compared with the broiler at day 28. There was a gradual decrease in the duration of TI ($F_{4,\,440} = 12.27$; $P < 0.0001$) and behavioral vigilance ($F_{4,\,440} = 7.551$; $P < 0.0001$) in the treatment groups as the doses of Bactofort® is increased from 0.25 g/kg of feed in group II to 0.55 g/kg of feed in group V (Tables 5 and 6).

Correlation between thermal environmental parameters and behavioral vigilance

At day 28 and 42, the relationship between induction trial attempts and DBT ($r = -0.3222$, $P > 0.05$ and $r = -0.4622$, $P > 0.05$) and THI ($r = -0.2957$, $P > 0.05$ and $r = -0.3001$, $P > 0.05$) were negative and insignificant in the control group. The relationship between DBT and THI were positive and stronger in the Bactofort®-treated groups at day 28 and 42, in comparison with the control group (Table 7).

Discussion

The thermal environmental parameters recorded inside the broiler chicken house were characterized by wide oscillation or variation in DBT, THI, and RH, classical of the cold-dry season in the tropical guinea savannah zone of northern Nigeria (Ayo et al., 2014; Dzenda et al., 2011; Zakari et al., 2018). The DBT value recorded at 13:00 h during the study period was outside the established thermoneutral zone of 12-24°C (Plyaschenko and Sidorov, 1987) and 20.9-28.5 °C (Prinzinger et al., 1991) in broiler chickens reared in the temperate and tropical/subtropical regions of the world, respectively. The results showed that the fluctuating thermal environmental parameters obtained in the broiler chicken house at 06:00 and 13:00 h were stressful to the broiler chicken and this may influence their behavioral responses. Similarly, the wide variation in RH at 06:00 h (70.17 ± 2.39 %) and 13:00 h (42 ± 1.81 %) obtained in this study were higher than the normal value of 30 to 40 %, established for broiler chickens raised in the tropical and subtropical regions of the world (Oluyemi and Roberts, 2000). The finding showed that the high DBT and RH are mainly responsible for the initiation of thermal stress resulting in longer duration of TI, low number induction trial attempts, and high behavioral vigilance in the control group. The THI which is an index of thermal stress recorded at 06:00 h (23.33 ± 0.67), 13:00 h (34.52 ± 0.17) and 19:00 h (24.60 ± 0.78) showed that the thermal environmental conditions in the broiler chicken house during the study period were not conducive for the rearing of broiler chickens in the northern guinea savannah zone of Nigeria. The finding of the present study agrees with the report of Purswell et al. (2012) that as THI increases above 21°C, body temperature of broiler chickens increases drastically to 1.7 °C above the normal body temperature of 41°C. As a result of the fluctuating and high DBT, RH, and THI obtained during the study period in the zone, measures aimed at ameliorating the impact of this adverse environmental condition are necessary to reduce the effect of thermal stress on the broiler chickens. The high DBT and RH reduce heat loss by evaporative cooling, which may result in thermal stress (Islam et al., 2013). An increase in ambient temperature above the thermoneutral zone in birds results in diversion of energy from production to activities responsible in the maintenance of homeothermy, which adversely affects productivity (Marai et al., 2002). Therefore, measures aimed at reducing the adverse effect of this unfavorable environmental condition in the poultry pen may enhance their productivity in the tropical guinea savannah zone of Northern Nigeria.

In the present study, there was profound impact of environmental stressors on fearfulness in the control group exhibited by long duration of TI, low number of induction trial attempts, and high behavioral vigilance. This is contrary to the findings of Zulkifli et al., (1999) who reported that environmental stressors do not influence the duration of TI. Variation in genetic background, age, and prior experiences could be responsible for the discrepancies in the results obtained. In previous studies, TI duration has been found to be useful in birds when measuring the effect of different and presumably stressful situations such as transportation and handling procedures, new environments, and

Table 5
Effects of age on tonic immobility duration (seconds) in broiler administered graded level of Bactofort® during the cold-dry season (n = 15)

Age	Group I (0)	Group II (0.25 g/kg)	Group III (0.35 g/kg)	Group IV (0.45 g/kg)	Group V (0.55 g/kg)	Age		Treatment		Interaction	
						P values	*F* values	*P* values	*F* values	*P* values	*F* values
Day 28	$147.91 \pm 17.25^{a,1}$	$143.62 \pm 15.9^{a,1}$	$112.40 \pm 12.22^{b,1}$	$101.47 \pm 11.25^{b,1}$	$85.38 \pm 9.60^{c,1}$	0.0402	7.551	< 0.0001	12.27	0.2673	0.2673
Day 42	$166.42 \pm 18.62^{a,1}$	$110.76 \pm 12.58^{b,2}$	$85.22 \pm 6.73^{c,2}$	$78.00 \pm 7.44^{d,2}$	$74.76 \pm 9.30^{d,1}$						

Values are expressed as mean ± SEM.
Group 1 = control, group II = 0.25 g/kg, group III =0.35 g/kg, group IV = 0.45 g/kg, and group V = 0.55 g/kg.
[a,b,d,e] Means for the same row having different superscript numbers are significantly ($P < 0.05$) different.
[1,2] Means for the same column having different superscript letters are significantly ($P < 0.05$) different.

Table 6
Effects of age on behavioral vigilance in broiler administered graded level of Bactofort® during the cold-dry season (n = 15)

Age	Group I (0)	Group II (0.25 g/kg)	Group III (0.35 g/kg)	Group IV (0.45 g/kg)	Group V (0.55 g/kg)	Age		Treatment		Interaction	
						P values	*F* values	*P* values	*F* values	*P* values	*F* values
Day 28	2.71 ± 0.07[a,1]	1.84 ± 0.078[b,1]	1.51 ± 0.09[c,1]	1.33 ± 0.07[d,1]	1.16 ± 0.06[e,1]	0.0005	12.25	< 0.0001	62.45	< 0.0001	12.60
Day 42	1.98 ± 0.08[a,2]	1.67 ± 0.08[b,2]	1.57 ± 0.08[c,1]	1.47 ± 0.08[d,2]	1.33 ± 0.07[e,2]						

Values are expressed as mean ± SEM.
Group 1 = control, group II = 0.25 g/kg, group III = 0.35 g/kg, group IV = 0.45 g/kg, and group V = 0.55 g/kg.
[a,b] Means for the same row having different superscript numbers are significantly ($P < 0.05$) different.
[1,2] Means for the same column having different superscript letters are significantly ($P < 0.05$) different.

also fear in social situations (Bilcik et al., 1998; Scott et al., 1998). Supplementation of *B. subtilis*–based probiotic reduces heat stress–related behaviors such as wing spreading, panting, squatting close to the ground, drinking, sleeping, dozing, and sitting (Wang et al., 2018).

Physiological stress responses and level of fearfulness are used to assess welfare of broiler chickens after they are subjected to stress (Nicol and Scott, 1990). The gradual increase in the dosage of Bactofort® decreases the duration of TI and behavioral vigilance indicating that the use of probiotics reduces fear. The results of the present study showed that the broiler chicken fed with 0.55 g/kg of Bactofort® recorded the shortest duration of TI and reduced behavioral vigilance, whereas the longest duration of TI and higher behavioral vigilance was observed in the control birds. This agrees with the findings of Skomorucha et al. (2010), who observed that broiler chickens subjected to thermal environmental stress displayed reduced welfare, evidenced by longer TI and behavioral vigilance, but supplementation with graded levels of Bactofort decreases the fear-induced stress response. Jones and Faure (1980) and Yildirim and Taskin (2017) reported that animals with longer TI were classified as being more passive and fearful, whereas those with shorter TI were evaluated as being more active and fearless. Although, Ghareeb et al. (2008) reported that probiotic-containing *Lactobacillus sp.* can modulate the physiological stress response of broiler chickens subjected to stress and consequently enhance tolerance to stress without change in their behavioral index of fear. Jahromi et al. (2016), Lara and Rostagno (2013), and Sohail et al. (2010) reported that dietary supplementation with lactobacillus-based probiotic can reduce some of the adverse effects of heat stress in broiler chicken.

Antipredator behavior such as TI and behavioral vigilance are used as indices of fearfulness and it duration indicates the level of stress in birds (Ghareeb et al., 2014). The result of the present study showed that the duration of TI at day 42 was longer than day 28 in the control group, which may suggest that fear response in birds increase with age in the group without Bactofort® supplementation. This agrees with the findings of Egbuniwe et al. (2016) who reported that the adverse effect of environmental conditions were mostly pronounced at day 42 of age, when they are expected to reach market weight. Both the duration of TI and the ease of being induced represent a useful behavioral index of fear (Faure and Mills, 1998). The duration of TI was considered to be a measure of the fear level of a bird immediately preceding its induction into TI and of the underlying fearfulness of a bird (Jones, 1996) under the assumption that the bird is more frightened and more fearful when TI is easily induced and remained immobile for longer duration (Scott and Moran, 1993). Fear is widely regarded as an undesirable state of suffering by the scientific community, welfare and policy groups, as well as farmers. It can be a powerful and potentially damaging stressor, especially if it is intense or persistent. High levels of underlying fearfulness have also been negatively correlated with growth, plumage condition, and food conversion efficiency Jones (1996). The result of the present study showed that the administration of different levels of Bactofort® in broiler chickens elicited boldness and confidence via freezing behavior suppression. The positive and relatively stronger correlation obtained between THI and TI induction attempts in the treated groups demonstrated that the broiler chickens were more active compared with the control group, apparently because of the wide variation of DBT and RH as well as the adverse effects of high DBT and RH in the broiler chickens during the cold-dry season.

Table 7
The relationships between thermal environment parameters and tonic immobility induction trial attempts of broiler chickens administered with graded level of Bactofort® during the cold-dry season from day 28-42 (n = 15)

Age, days	Correlated parameters	Groups				
		Group 1 (ctrl)	Group II (0.25 g/kg)	Group III(0.35 g/kg)	Group 1V(0.45 g/kg)	Group V(0.55 g/kg)
28	TI induction attempts and DBT	−0.3222	0.3247	0.2818	0.1887	0.4990
	TI induction attempts and THI	−0.2957	0.3187	0.2594	0.4499	0.4570
	TI induction attempts and RH	0.1722	0.2179	0.1296	0.2621	0.1308
42	TI induction attempts and DBT	−0.4622	0.7074	0.7494[a]	0.4796	0.7116[b]
	TI induction attempts and THI	−0.3001	0.4236	0.7388[b]	0.2008	0.7154[a]
	TI induction attempts and RH	0.3585	0.1358	0.3435	0.4204	0.8155[c]
Overall	TI induction attempts and DBT	−0.4550	0.3340	0.4560	0.1678	0.5122[a]
	TI induction attempts and THI	−0.3550	0.2220	0.5012	0.3221	0.6011[a]
	TI induction attempts and RH	−0.2334	0.2150	0.3331	0.3122	0.4576

Group 1 = control, group II = 0.25 g/kg, group III = 0.35 g/kg, group IV = 0.45 g/kg, and group V = 0.55 g/kg.
DBT, dry-bulb temperature; RH, relative humidity; TI, tonic immobility; THI, temperature-humidity index.
[a] $P < 0.05$, significant correlation.
[b] $P < 0.01$, very significant correlation.
[c] $P < 0.001$.

Conclusion

In conclusion, the administration of probiotics decreases the duration of TI and behavioral vigilance in the treatment groups as the doses of Bactofort® is increased from 0.25 g/kg of feed in group II to 0.55 g/kg of feed in group V. Thus, the use of graded levels of Bactofort® reduced stress response and may be beneficial in the improvement of health and welfare of broiler chickens during the Harmattan season.

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Ethical consideration

The management system adhered to the new European Union (EU) council directive 2007/43/EC of laid down minimum rules for the protection of chickens, kept for meat production (European Commission, 2007).

Conflict of interest

The authors declare that they have no conflicts of interest.

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