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Perinatal Thermal Conditioning of Three Broiler Strains in Southwestern Nigeria

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ABSTRACT

Background and Objective: Heat stress resulting from climate change has increasingly challenged the sustainability of poultry production in the tropics. This study determined the effect of early-age thermal conditioning in selected local and exotic chickens in Nigeria. **Materials and Methods:** On day six, twenty chicks each from Cobb 500 (C500), Ross 308 (R308) and improved Nigerian indigenous broiler-FUNAAB Alpha (FA) strains were thermally conditioned at 40±1°C for 3 hrs. Conditioned and unexposed chicks were acutely challenged at 40±1°C for 15 min on day ten, just before collecting blood and tissue samples for haematology and qPCR, respectively. **Results:** Thermal conditioning significantly ($p<0.05$) lowered all heat stress indices in this study. Significance ($p<0.05$) was observed on haematological parameters and BDNF gene expression by interactive effect of strain and thermal conditioning. The highest means for packed cell volume, haemoglobin and red blood cell counts were recorded in conditioned C500 while conditioned FA had the highest expression of BDNF. **Conclusion:** This study showed that response to perinatal heat conditioning in chickens is strain-specific. To tackle climate change effects in the southwestern part of Nigeria and generally, in the tropics, it is recommended that farmers thermally condition commercial broiler chicks.

KEYWORDS

Tropics, anterior hypothalamus, brain-derived neurotrophic factor, global warming, qPCR, strain-specific, thermo-tolerance, thermoregulation

INTRODUCTION

Global warming has aggravated heat stress-related problems in poultry production[1]. While demand for livestock products is expected to continue to increase globally[2,3], the growth and meat quality of broilers has been negatively affected by heat stress[4].

Chicken thermoregulation, on which individual bird's response to thermal stress depends[5-8] has been revealed to be heavily influenced by genetic or strain differences.

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Acquisition of thermo-tolerance, which enables broiler chickens to adapt to extreme heat due to climate change, has been found to be enhanced by thermal conditioning, which is achieved by exposing poultry species to high ambient temperatures during critical periods[9].

It has been verified that the Brain-Derived Neurotrophic Factor activates the pathway that leads to the establishment of thermal stress response threshold in birds. Also, short-term differences are observable in the expression of the gene during both heat conditioning and re-exposure of conditioned chicks to heat stress, when compared to its expression in unconditioned chicks of the same age[10-11]. It has been reported that memory, adaptation and survival are improved by higher expressions of the BDNF gene[12-13].

Farmers raising commercial broilers, especially in the tropical regions of the world need guidance in making profitable strain or breed selection and mitigation of the effects of heat stress due to climate change. Information on the verification of the interactive effect of strain and thermal treatment at critical periods on thermoregulation and thermo-tolerance of individual chickens was not available as of when this study was conducted, to the best of the author's knowledge. This study, therefore, determined the effect of perinatal thermal conditioning on heat stress indices, haematological parameters and expression of Brain-Derived Neurotrophic Factor (BDNF) gene in Cobb 500 (C500), Ross 308 (R308) and improved Nigerian indigenous broiler-FUNAAB Alpha (FA).

MATERIALS AND METHODS

Experimental birds: Fifty days old chicks each of Cobb 500 and Ross 308, which were obtained from Zartech Farms and Agrited Nigeria Limited, respectively in Ibadan, as well as the improved Nigerian indigenous broiler-FUNAAB Alpha, obtained from the Federal University of Agriculture, Abeokuta (FUNAAB) hatchery, Nigeria. For the ten days of the experiment, which was conducted in November, 2016, they were provided with a conventional starter mash (23% crude protein, 2100 kcal metabolisable energy) and water *ad libitum*.

Experimental location: The field study was conducted in Abeokuta, Nigeria. The experimental birds were raised in partitioned brooder cages at an environmental temperature of 32±2°C and relative humidity of 70-80% under continuous artificial lighting[14-15]. Benchwork was carried out at Africa Biosciences, Ibadan, Nigeria.

Thermal conditioning and acute thermal challenge: On day six, chicks from each strain were randomly distributed into two treatment groups (conditioned and unconditioned) of twenty-five birds each. While the unconditioned group were left at normal brooding temperature, the conditioned group were exposed to high temperature at 40±1°C for 3 hrs. This study adopted day 6 for thermal conditioning based on reports by researchers[16,17]. On day ten, both groups were exposed to acute thermal challenge at 40±1°C for 15 min without feed and water[17].

Data collection: Rectal temperatures and respiratory rates of each bird (before and immediately after the acute heat challenge) were measured using a digital thermometer and stopwatch, respectively.

Immediately after the acute heat challenge, about 2 mL of blood was collected from ten birds from each group for all three strains via cardiac puncture[17]. The blood samples were dispensed into clean bijou bottles with Ethylenediaminetetraacetic Acid (EDTA) as an anticoagulant, for determining the Packed Cell Volume (PCV), Haemoglobin concentration (Hb), Red Blood Cell (RBC) and white blood cell differential (heterophils, lymphocytes, basophils and eosinophils) counts.

Table 1: Primer sequences used for quantitative real-time PCR in this study

Gene	Forward primer (F)	Reverse primer (R)
BDNF	5'-TGGGTAACAGCAGCGGAGAA-3'	5'-TATTGCTTCAGTTGGCCTTTAG-3'
GAPDH	5'-AAGGAGTGAGCCAAGCACACA-3'	5'-TCACTGCAGGATGCAGAACTG-3'
β-Actin	5'-CCCAAAGCCAACAGAGAGAAG-3'	5'-ACCATCACCAGAGTCCATCAC-3'

BDNF: Brain-derived neurotrophic factor, GAPDH: Glycealdehyde-3-phosphate dehydrogenase and Source: Byerly *et al.*[18]

Also, from each strain, five random chicks each from the conditioned and unconditioned groups were slaughtered by cervical dislocation. From the anterior hypothalamus of each bird, 0.5 g tissue samples were collected and stored immediately in Eppendorf tubes containing 2.5 µL RNA later solution (i.e. 1:5) and stored at -40°C.

Total RNA isolation from each stored sample was carried out using Norgen's Animal Tissue RNA purification kit while Norgen's TruScript™ First Strand cDNA Synthesis Kit was used for the First-Strand cDNA Synthesis reaction. The Norgen products, including the RNA later solution, were supplied by Norgen Biotek Corp., Thoroid, Ontario, Canada.

The real-time polymerase chain reaction of cDNA products was performed using the Cepheid SmartCycler, manufactured by Cepheid, Sunnyvale, California, USA. Relative quantitation of the cDNA for the BDNF gene was carried out using a 5X EvaGreen master mix (manufactured by Solis Biodyne) containing DNA Polymerase, dNTPs, $MgCl_2$ and EvaGreen dye. The total reaction mixture of 20 µL contained 4 µL master mix, 0.3 µL forward and reverse primers (Table 1), 2 µL template and 13.4 µL PCR grade water.

Based on the result of an initial evaluation using gel electrophoresis, β-Actin was selected as the endogenous control gene. In compliance with the recommendation of the kit manufacturer, in order to activate the DNA polymerase in the master mix, an initial incubation step of 12 min at 95°C was carried out at the beginning of the qPCR cycle. Following this were 40 cycles of three qPCR steps of denaturation at 95°C for 15 sec, annealing (calculated for each primer from their melting temperatures) for 20 sec and extension at 72°C for 20 sec. Samples were run in duplicates and the averages were recorded to enhance the reliability of the results as shown in Table 1.

Statistical analysis: To relatively quantify BDNF gene expression, the ΔCT value for each sample was obtained by subtracting the CT value of β-actin (reference gene) from the CT of BDNF (target gene). The ΔΔCT was generated by subtracting ΔCT for the control group from ΔCT of each experimental sample[19]. The approximate fold difference, $2^{-\Delta\Delta CT}$ values, were calculated using the ΔΔCT.

Data obtained for heat stress and haematological parameters as well as the approximate fold difference ($2^{-\Delta\Delta CT}$) values from gene expression data were analyzed using the General Linear Model of SAS 9.0. Duncan's Multiple Range Test was used for means separation[20] and the results presented as Means±Standard Error (SE), at a 5% level of significance.

RESULTS

The effect of strain was significant on rectal temperature before acute heat exposure (RT1), rectal temperature after acute heat challenge (RT2), respiratory rate before acute heat challenge (RR1) and respiratory rate after acute heat challenge (RR2) as shown in Table 2. Thermal conditioning significantly ($p<0.05$) lowered all heat stress parameters in this study. Comparison among the six treatments resulting from the combination of the strain and thermal conditioning showed significant ($p<0.05$) differences in the four parameters.

The haematological profile of the three strains were statistically similar. Thermal conditioning significantly ($p<0.05$) increased heterophil/lymphocyte ratio (H/LR) (Table 3).

Table 2: Effects of chicken strain, thermal conditioning and the interaction of strain and thermal conditioning on heat stress parameters (Mean±SE)

Parameter	FA	C500	R308
RT1 (°C)	41.200±0.093[a]	41.850±0.120[a]	41.900±0.078[a]
RT2 (°C)	42.700±0.109[b]	43.150±0.18[a]	43.100±0.105[a]
RR1 (counts min^{-1})	97.600±1.122[b]	111.500±1.132[a]	111.800±1.122[a]
RR2 (counts min^{-1})	114.400±1.046[b]	124.100±1.348[ab]	131.000±1.463[a]
Parameter	Conditioned		Unconditioned
RT1 (°C)	41.302±0.059[b]		41.500±0.164[a]
RT2 (°C)	42.442±0.070[b]		42.940±0.069[a]
RR1 (count min^{-1})	101.938±0.717[b]		104.280±0.709[a]
RR2 (count min^{-1})	117.129±0.669[b]		126.080±0.662[a]

Parameter	FA CON	FA UNC	C CON	C UNC	R CON	R UNC
RT1 (°C)	41.10±0.16[b]	41.20±0.16[b]	41.58±0.13[ab]	41.77±0.13[a]	41.65±0.13[ab]	41.70±0.13[a]
RT2 (°C)	42.00±1.12[b]	42.70±1.12[ab]	42.81±0.18[ab]	43.29±0.18[a]	42.82±1.15[ab]	43.31±1.15[a]
RR1 (count min^{-1})	96.40±7.16[c]	98.80±7.15[c]	111.00±8.15[a]	112.00±8.15[a]	111.0±10.16[a]	112.60±10.2[a]
RR2 (count min^{-1})	110.0±10.15[d]	118.80±10.15[c]	117.0±11.15[c]	131.20±9.45[a]	128.2±10.2[ab]	133.80±14.2[a]

[a,b,c]Mean along the same row with different superscripts are significantly different at the 5% ($p<0.05$) level, RT1: Rectal temperature before acute heat exposure, RT2: Rectal temperature after acute heat challenge, RR1: Respiratory rate before acute heat challenge, RR2: Respiratory rate after acute heat challenge, C: 500, R: R308, CON: Conditioned and UNC: Unconditioned

Table 3: Effect of chicken strain and thermal conditioning on haematological parameters (Mean±SE)

Parameter	FA	C500	R308
PCV	33.333±2.43	31.300±2.545	29.700±2.545
HB	11.040±0.806	10.410±0.842	9.950±0.842
RBC	2.525±0.188	2.352±0.196	2.255±0.196
HET	37.833±1.688	39.600±1.763	36.400±1.763
LYM	58.250±1.555	56.700±1.624	59.700±1.624
H/LR	0.658±0.049	0.713±0.051	0.624±0.051
EOS	3.250±0.311	2.800±0.325	2.900±0.325
MCV	13.200±1.018	13.191±0.618	13.130±0.225
MCHC	0.332±0.006	0.333±0.004	0.336±0.004
MCH	4.356±0.283	4.394±0.164	4.407±0.047
Parameter		Conditioned	Unconditioned
PCV		31.707±1.555	28.200±1.610
HB		10.567±0.515	9.428±0.533
RBC		2.334±0.120	2.140±0.124
HET		41.060±1.077[a]	36.760±1.115[b]
LYM		55.680±0.992[b]	59.360±1.027[a]
H/LR		0.755±0.031[a]	0.629±0.032[b]
EOS		2.693±0.199	2.800±0.20
MCV		14.379±4.9283	13.111±0.697
MCHC		0.333±0.0041	0.335±0.0051
MCH		4.793±1.6408	4.393±0.2143

[a,b]Mean along the same row with different superscripts are significantly different at the 5% ($p<0.05$) level, PCV: Packed Cell Volume (%), HB: Haemoglobin (g dL^{-1}), RBC: Red Blood Cell (%), HET: Heterophil (%), LYM: Lymphocytes (%), H/LR: Heterophil/lymphocyte ratio, EOS: Eosinophil (%), MCV: Mean corpuscular volume, MCHC: Mean corpuscular haemoglobin and MCH: Mean corpuscular haemoglobin concentration

The interactive effect of strain and thermal conditioning was significant ($p<0.05$) on all haematological parameters was shown in Table 4.

The BDNF gene was most expressed in FA and least expressed in C500 strain (Fig. 1a). Thermal conditioning significantly ($p<0.05$) increased BDNF expression (Fig. 1b). The interactive effect of strain and thermal conditioning was also significant ($p<0.05$), with unconditioned C500 having the least and conditioned FA the highest expression of BDNF gene (Fig. 1c). However, there was no statistical difference between conditioned and unconditioned FA.

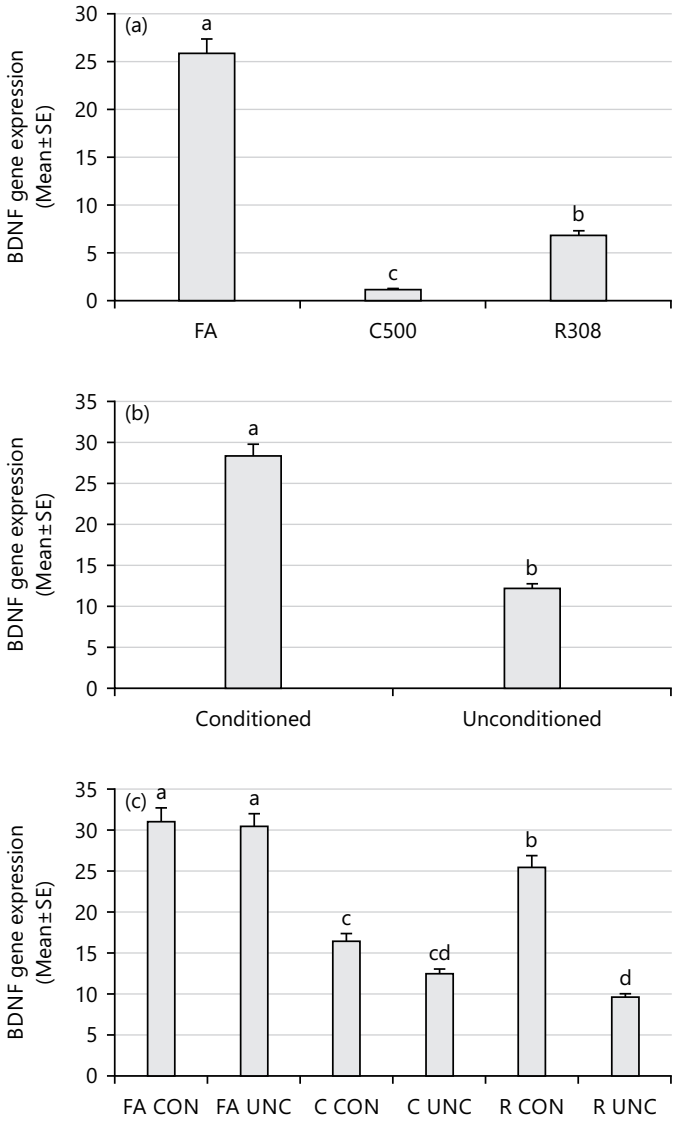

Fig. 1(a-c): Effect of (a) Chicken strain, (b) Thermal conditioning and (c) Strain×thermal conditioning interaction on relative expression of BDNF Gene

[a,b,c,d]Means with different superscripts are significantly different at the 5% ($p<0.05$) level, FA: FUNAAB alpha, C: C500, R: R308, CON: Conditioned and UNC: Unconditioned

Table 4: Interactive effect of strain and thermal conditioning on haematology (Mean±SE)

Parameter	FA CON	FA UNC	C CON	C UNC	R CON	R UNC
PCV	33.67±2.43^{ab}	33.00±2.32^{ab}	37.20±2.48^{a}	25.40±2.33^{b}	25.40±2.33^{b}	26.00±2.58^{b}
HB	11.10±0.796^{ab}	10.98±0.832^{ab}	12.34±0.716^{a}	8.48±0.806^{b}	8.48±0.806^{b}	8.68±0.806^{b}
RBC	2.47±0.208^{b}	2.58±0.223^{b}	2.78±0.223^{a}	1.92±0.223^{bc}	1.92±0.223^{bc}	1.96±0.223^{bc}
HET	37.66±2.32^{b}	38.00±2.41^{b}	43.00±2.32^{ab}	36.20±2.34^{bc}	36.20±2.34^{bc}	35.00±2.34^{c}
LYM	58.50±1.624^{ab}	58.00±1.555^{b}	53.40±1.555^{bc}	60.00±1.624^{a}	60.00±1.624^{a}	60.60±1.624^{a}
HLR	0.65±0.037^{ab}	0.66±0.039^{ab}	0.82±0.03^{a}	0.61±0.029^{b}	0.61±0.029^{b}	0.60±0.032^{b}
EOS	3.50±0.31^{a}	3.00±0.23^{ab}	2.40±0.23^{b}	3.20±0.23^{ab}	3.20±0.23^{ab}	3.00±0.23^{ab}
MCV	13.69±0.335^{b}	12.6±0.335^{b}	13.39±0.335^{b}	12.99±0.345^{b}	12.99±0.345^{b}	13.14±0.433^{b}
MCHC	0.333±0.03^{ab}	0.329±0.02^{b}	0.332±0.11^{ab}	0.34±0.03^{a}	0.34±0.03^{a}	0.34±0.03^{a}
MCH	4.50±0.04^{b}	4.21±0.04^{b}	4.44±0.133^{b}	4.35±0.133^{b}	4.35±0.133^{b}	4.4±0.061^{b}

[a,b,c]Mean along the same row with different superscripts are significantly different at the 5% ($p<0.05$) level, PCV: Packed Cell Volume (%), HB: Haemoglobin (g dL^{-1}), RBC: Red Blood Cell (%), HET: Heterophil (%), LYM: Lymphocytes (%), H/LR: Heterophil/lymphocyte ratio, MONO: Monocytes (%), EOS: Eosinophil (%), MCV: Mean corpuscular volume, MCH: Mean corpuscular haemoglobin MCHC: Mean corpuscular haemoglobin concentration, FA: FUNAAB alpha, NF: Normal feather indigenous, C: C500, R: R308, CON: Conditioned and UNC: Unconditioned

DISCUSSION

As revealed by heat stress parameters, FA was less stressed than the two exotic strains. This is likely due to genetic differences in heat tolerance of the chicken strains, FA being a local strain. In the tropics, under the same environmental conditions, indigenous chickens have been reported to adapt and resist diseases better than the exotics[21,22]. The reduction in heat stress parameters observed in the conditioned chicks could have been due to acclimation while the sharp increase in heat stress experienced by unconditioned chicks was due to the acute heat challenge, for which conditioned chicks were already prepared. The interactive effect further confirms that thermal conditioning alleviated the effect of acute heat exposure, especially for the exotic strains (C500 and R308). According to Kennedy *et al.*[23], perinatal exposure of poultry to high environmental temperatures improves their thermo-tolerance.

Though the blood profiles of the three strains used in this study were revealed to be statistically similar, only the FA strain mean values for important erythrocytic and leukocytic indices fall within reference ranges[24]. PCV, Hb, RBC and EOS levels being higher in FA strain could indicate better health status since the transport of oxygen and absorbed nutrients involve PCV and Hb[25,26]. Thermal conditioning increased PCV, Hb and RBC in the two commercial strains (C500 and R308), whereas it had no effect on FA. This study has also shown that the effect of strain by thermal conditioning interaction (i.e., genotype×environment) on haematological parameters is strain-specific. This result agreed with Siegel[27], who reported that various lines exhibited different responses in the same environmental conditions because of the genotype×environment interaction. Meanwhile, haematological parameters have been suggested for use in the selection of animals that are genetically resistant to certain environmental conditions[25,28,29].

The BDNF gene is essential for synaptic plasticity and maintenance of long-term memory[30], its expression has been found to be higher in thermally conditioned chicks in comparison with the unconditioned[11]. The difference in the expression of the gene in the three strains further affirms strain differences in chicken thermoregulation, which affects how individual birds respond to thermal stress[7,8]. The higher expression of the BDNF gene in conditioned birds agrees with the findings of Labunskay and Meiri[10] and Yossifoff *et al.*[11] and confers greater developmental plasticity which could make the thermally conditioned birds more thermo-tolerant when exposed to heat stress at marketing age[12,13]. However, the interactive effect of strain and thermal treatment suggests that the effect of perinatal thermal treatment is strain-specific since the improved indigenous strain had the highest expression of the BDNF gene and showed no statistical difference in the conditioned and unconditioned treatments. This possibly also explains why it has lower values for heat stress parameters which are indicators of thermal stress.

Based on this study, thermal conditioning of chickens is recommended for the alleviation of heat stress, especially in commercial broilers to be raised in Nigeria. This will help farmers to avoid losses and maximize profit in the face of increasing climate change effects. The molecular aspect of this study faced the challenges of limited funding, equipment and reagents. The number of chicken strains assessed was also limited. Hence, it is recommended that global gene expression profiling of thermoregulatory genes be carried out for a more robust assessment of thermal conditioning in tropical chicken strains.

CONCLUSION

Early-age thermal conditioning is beneficial for alleviating thermal stress in broiler or meat-type chickens raised in hot climates. In this study, the improved indigenous strain had an edge over exotic commercial strains in terms of heat tolerance. However, thermal conditioning enhanced thermo-tolerance in all the strains.

SIGNIFICANCE STATEMENT

This study was conducted to determine the effect of perinatal thermal conditioning on heat stress indices, haematological parameters and expression of the Brain-Derived Neurotrophic Factor (BDNF) gene in commercial broiler chicken strains in Nigeria. To the authors' best knowledge, this information was unavailable at the time of conducting the research. The study showed that perinatal thermal conditioning reduced the stress indices measured and is therefore recommended to help farmers mitigate the effects of climate change in tropical regions.

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