

Posthatch Thermal Conditioning Reduces Heat Stress In Three Broiler Strains

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Abstract

Heat stress is an increasing challenge to the sustainability of poultry production in the tropics due to global warming. This study determined the effect of posthatch thermal conditioning on heat stress indices, haematological parameters and expression of brain derived neurotrophic factor (BDNF) gene in three meat type chickens; Cobb 500 (C500), Ross 308 (R308) and improved Nigerian indigenous broiler - FUNAAB Alpha (FA). The interplay of individual bird's genetics and thermal treatment at critical periods on thermoregulation was largely unpublished as at the time this study was conducted. Thermal conditioning was carried out on day 6 by exposing 20 chicks from each strain to high temperature of 40 ± 1 °C for 3 hours. Both conditioned and unconditioned chicks were exposed to acute heat challenge of 40 ± 1 °C for 15 minutes on day 10. Blood samples were collected to determine haematological parameters. Tissue samples were collected from which RNA were extracted, synthesized into cDNA and subjected to qPCR. Strain and thermal conditioning interaction was significant ($p < 0.05$) on haematological parameters with conditioned C500 having the highest means for packed cell volume, haemoglobin and red blood cell counts. Interactive effect was also significant ($p < 0.05$) on BDNF gene expression, with conditioned FA having the highest. The study concluded that variation in traits due to thermal treatment is strain-specific and thermal conditioning is recommended for commercial broilers in southwestern Nigeria.

Introduction

Climatic stress has been described as the limiting factor to animal production efficiency and global warming will further accentuate heat stress related problems (Renaudeau et al., 2011). United Nations statistics projects increase in human population by over two billion by 2050 (UN, 2013). Global demand for livestock products is also expected to double (Rojas-Downing et al., 2017). Heat stress is a major challenge against poultry production worldwide, causing decline in growth and meat quality of broilers (Lara and Rostagno, 2013).

Rajkumar et al. (2010) and Fathi *et al.* (2013) reported that genetics contribute substantially to the response of birds to high temperature. Strain differences have been found to play a major role in chicken thermoregulation, which in turn determine how individual birds respond to thermal stress (Altan et al., 2003; Nielsen et al., 2003).

Thermal conditioning, which is the exposure of poultry species to high ambient temperature during critical periods has been found to improve the acquisition of thermo-tolerance (De Basilio et al., 2001; Yahav and Hurwitz, 1996; Yahav and McMurtry, 2001) enabling broilers to cope with extreme environmental conditions.

It has been demonstrated that the pathway leading to thermal stress response set-point establishment is activated by Brain-Derived Neurotrophic Factor and there are transient changes in its expression during both thermal conditioning and re-exposure of conditioned chicks to heat stress, compared to unconditioned chicks of the same age (Katz and Meiri, 2006; Labunskay and Meiri, 2006; Tirosh *et al.*,

2007; Yossifoff et al., 2008). Higher expression of the BDNF gene, which plays a pivotal role in developmental plasticity, has been found to improve memory, adaptation and survival (Johnston et al., 1999; Johnston and Rose, 2001).

However, the interplay of individual bird's genetics and thermal treatment at critical periods on thermoregulation was largely unpublished as at the time this study was conducted. This study was therefore aimed at verifying the interactive effect of strain and thermal conditioning on thermotolerance, to help farmers, especially in the tropics, in breed selection to alleviate the effects of heat stress in raising commercial broilers. Effect of posthatch thermal conditioning on heat stress indices, haematological parameters and expression of brain derived neurotrophic factor (BDNF) gene in three meat type chickens; Cobb 500 (C500), Ross 308 (R308) and improved Nigerian indigenous broiler - FUNAAB Alpha (FA) was determined.

Materials And Methods

Experimental birds

Fifty chicks each of Cobb 500 (Cobb) obtained from Zartech Farms, Oluyole, Ibadan; Ross 308 (Ross) obtained from Agrited Nigeria Limited, Alomaja Ibadan and improved Nigerian indigenous broiler - FUNAAB ALPHA (FA) obtained from FUNAAB Hatchery was used for the study. The birds were placed into partitioned brooder cages at day old. They were raised in an adequate environmental temperature (32 ± 2 °C) under continuous artificial illumination and relative humidity of 70-80% (Gan *et al.*, 2013; Yahav, 2001) until day six. The experimental birds were fed with conventional starter mash with 23% crude protein and 2100 kcal metabolisable energy throughout the experimental period of ten days. Feed and water were provided *ad libitum*.

Thermal conditioning and acute thermal challenge

On day six, birds from each strain were randomly distributed into two treatment groups of twenty-five birds each. One group of chicks (thermally conditioned chicks) were exposed to high temperature at 40 ± 1 °C for 3 hours while the other group (unconditioned chicks) were left at normal brooding temperature. The age for the thermal conditioning (Day 6) applied in this study was adopted from previous reports (De Basilio et al., 2001, 2003; Tanizawa *et al.*, 2015). On day ten, both groups (conditioned and unconditioned chicks) were challenged to high temperature at 40 ± 1 °C for 15 minutes without feed and water (Tanizawa *et al.*, 2015).

Data Collection

Rectal Temperature and Respiratory Rate

Rectal temperature of each bird was measured by inserting a digital thermometer into its rectum. The reading was taken when it became stable and thermometer gave an alarm signal (Plyaschenko and

Sidorov, 1987). Respiratory rate was measured by counting the movement of the abdominal region or vent per minute using a stopwatch (Plyaschenko and Sidorov, 1987).

Blood Sampling

Immediately after the 15 minutes of acute heat exposure on day ten, blood samples were collected from ten heat challenged birds from each group for all the five strains by cardiac puncture (Tanizawa, 2015). About 2 ml of blood was collected from the heart of each bird and dispensed into clean *Bijou* bottle containing ethylene diamine tetra acetic acid (EDTA) as an anticoagulant. The un-coagulated blood was used to determine the Packed Cell Volume (PCV), haemoglobin concentration (Hb), red blood cell (RBC) and white blood cell differentials (heterophils, lymphocytes, basophils and eosinophils) counts.

Tissue Sampling

Ten chicks per breed across the two treatment groups (conditioned and unconditioned) were randomly selected and slaughtered by cervical dislocation. Tissue samples were collected from the anterior hypothalamus and immediately stored in eppendorf tubes containing RNAlater solution in the ratio of 1:5 i.e. 0.5g of tissue to 2.5 µl solution and kept at -40 °C till needed for further analyses.

Extraction of Total RNA and cDNA Synthesis

Norgen's Animal Tissue RNA purification kit was used to isolate Total RNA from the stored samples following the manufacturer's instructions. The First-Strand cDNA Synthesis reaction was carried out using Norgen's TruScript™ First Strand cDNA Synthesis Kit.

Real Time Quantitative Polymerase Chain Reaction (Real Time qPCR)

The cDNA products were subjected to real time PCR performed using the Cepheid SmartCycler. Relative quantitation of the cDNA for BDNF gene was carried out using 5X EvaGreen master mix (manufactured by Solis Biodyne) containing DNA Polymerase, dNTPs, MgCl₂ and EvaGreen dye. 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.

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'-TCACTGCAGGATGCAGAAGT-3'
β-Actin	5'-CCCAAAGCCAACAGAGAGAAG-3'	5'-ACCATCACCAGAGTCCATCAC-3'

BDNF - Brain-Derived Neurotrophic Factor; **GAPDH** -Glycealdehyde-3-Phosphate Dehydrogenase. Source: Byerly *et al.* (2009).

An initial incubation step of 12 min at 95°C was carried out at the beginning of the qPCR cycle to activate the DNA polymerase in the master mix as recommended by kit manufacturer. Thereafter followed by 40 cycles of three qPCR steps of denaturation at 95°C for 15 seconds, annealing (calculated for each primer from their melting temperatures) for 20 seconds and extension at 72°C for 20 seconds. After preliminary test, β -Actin was selected as the endogenous control to normalize for the amount of RNA added to the reverse transcription reactions.

Data Analyses

For relative quantification of BDNF gene expression, Δ CT value was calculated for each sample by subtracting CT value of β -actin (reference gene) from CT of BDNF (target gene). Δ CT for the control group was subtracted from the Δ CT of each experimental sample to generate $\Delta\Delta$ CT. Mathematically, $\Delta\Delta$ Ct = (CT, Target - CT, β Actin) experimental group - (CT, Target - CT, β Actin) control group (Livak and Schmittgen, 2001). The $\Delta\Delta$ CT was used to calculate the approximate fold difference, $2^{-\Delta\Delta$ CT} values.

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 analysed using the General Linear Model of SAS 9.0. Duncan Multiple Range Test was used to separate the means that differed significantly (Gomez and Gomez, 1984) and the result is presented as means \pm standard error (S.E).

Results

Heat Stress Parameters

The effect of strain was significant on heat stress parameters [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)]. FA had the lowest means in RR2 (114.400). R308 had highest mean values for RT1, RR1 and RR2 (1.900°C, 111.800 counts/min and 131.000 counts/min respectively). There was no difference in RT1 for the three strains but C500 and R308 had significantly ($p < 0.05$) higher means for RT2, RR1 and RR2 than FA (Table 2).

Table 2
Effect of Strain on Heat Stress Parameters (Means \pm S.E)

Parameters	FA	C500	R308
R.T1 ($^{\circ}$ C)	41.200 \pm 0.093 ^a	41.850 \pm 0.120 ^a	41.900 \pm 0.078 ^a
R.T2 ($^{\circ}$ C)	42.700 \pm 0.109 ^b	43.150 \pm 0.18 ^a	43.100 \pm 0.105 ^a
R.R1 (counts/min)	97.600 \pm 1.122 ^b	111.500 \pm 1.132 ^a	111.800 \pm 1.122 ^a
R.R2 (counts/min)	114.400 \pm 1.046 ^b	124.100 \pm 1.348 ^{ab}	131.000 \pm 1.463 ^a
^{a,b} Means along the same row with different superscripts are significantly different at 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			

Thermal conditioning significantly (p<0.05) lowered all heat stress parameters in this study (Table 3).

Table 3
Effect of Thermal Conditioning on Heat Stress Parameters (Means \pm S.E)

Parameters	Conditioned	Unconditioned
R.T1 ($^{\circ}$ C)	41.302 \pm 0.059 ^b	41.500 \pm 0.164 ^a
R.T2 ($^{\circ}$ C)	42.442 \pm 0.070 ^b	42.940 \pm 0.069 ^a
R.R1(count/min)	101.938 \pm 0.717 ^b	104.280 \pm 0.709 ^a
R.R2(count/min)	117.129 \pm 0.669 ^b	126.080 \pm 0.662 ^a
^{a,b} Means along the same row with different superscripts are significantly different at 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.		

The interactive effect of strain and thermal conditioning on RT1, RT2, RR1 and RR2 is presented in Table 4. Comparison among the six treatments resulting from the combination of the two factors showed significant (p<0.05) differences in the four parameters. The result showed that RT1 value varied from 40.680 $^{\circ}$ C to 41.770 with the highest value recorded in Unconditioned C500 while the least value was recorded in Conditioned FA. RT2 values varied from 41.630 $^{\circ}$ C to 43.310 $^{\circ}$ C with the highest value recorded in Unconditioned R308 while the least value was recorded in Conditioned FA. RR1 values varied from

93.400 counts/min to 112.600 counts/min with the highest value recorded in Unconditioned R308 while the least value was recorded in Conditioned FA. RR2 values varied from 110.000 counts/min to 133.800 counts/min with the highest value recorded in Unconditioned R308 while the least value was recorded in Conditioned FA.

Table 4

Effect of the Interaction of Strain and Thermal Conditioning on Heat Stress Parameters (Means \pm S.E)

Parameters	FA CON	FA UNC	C CON	C UNC	R CON	R UNC
R.T1 (°C)	41.10 \pm 0.16 ^b	41.20 \pm 0.16 ^b	41.58 \pm 0.13 ^{ab}	41.77 \pm 0.13 ^a	41.65 \pm 0.13 ^{ab}	41.70 \pm 0.13^a
R.T2 (°C)	42.00 \pm 1.12 ^b	42.70 \pm 1.12 ^{ab}	42.81 \pm 0.18 ^{ab}	43.29 \pm 0.18^a	42.82 \pm 1.15 ^{ab}	43.31 \pm 1.15 ^a
R.R1 (c/min)	96.40 \pm 7.16 ^c	98.80 \pm 7.15 ^c	111.00 \pm 8.15 ^a	112.00 \pm 8.15 ^a	111.0 \pm 10.16 ^a	112.60 \pm 10.2^a
R.R2 (c/min)	110.0 \pm 10.15^d	118.80 \pm 10.15 ^c	117.0 \pm 11.15 ^c	131.20 \pm 9.45 ^a	128.2 \pm 10.2 ^{ab}	133.80 \pm 14.2^a
C = C500, R = R308, CON = Conditioned, UNC = Unconditioned, c/min = count/min						

Haematological parameters

The haematological profile [packed cell volume (PCV), Haemoglobin count (HB), Red Blood Cell (RBC), Heterophil count (HET), Lymphocyte count (LYM), Heterophil-Lymphocyte Ratio (H/LR), eosinophils (EOS), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC)] of the three strains were statistically similar (Table 5).

Table 5
Effect of Chicken Strain on Haematological Parameters (Means \pm S.E)

Parameters	FA	C500	R308
PCV	33.333 \pm 2.43	31.300 \pm 2.545	29.700 \pm 2.545
HB	11.040 \pm 0.806	10.410 \pm 0.842	9.950 \pm 0.842
RBC	2.525 \pm 0.188	2.352 \pm 0.196	2.255 \pm 0.196
HET	37.833 \pm 1.688	39.600 \pm 1.763	36.400 \pm 1.763
LYM	58.250 \pm 1.555	56.700 \pm 1.624	59.700 \pm 1.624
H/LR	0.658 \pm 0.049	0.713 \pm 0.051	0.624 \pm 0.051
EOS	3.250 \pm 0.311	2.800 \pm 0.325	2.900 \pm 0.325
MCV	13.200 \pm 1.018	13.191 \pm 0.618	13.130 \pm 0.225
MCHC	0.332 \pm 0.006	0.333 \pm 0.004	0.336 \pm 0.004
MCH	4.356 \pm 0.283	4.394 \pm 0.164	4.407 \pm 0.047
PCV: Packed Cell Volume (%), HB: Haemoglobin (g/dL), RBC: Red Blood Cell (%), HET: Heterophil (%), LYM: Lymphocytes (%), H/LR: Heterophil/Lymphocyte Ratio, EOS: Eosinophil (%), MCV: Mean Corpuscular volume, MCHC: Mean Corpuscular Haemoglobin MCH: Mean Corpuscular Haemoglobin Concentration.			

Thermal conditioning did not significantly ($p < 0.05$) influence PCV, RBC, HB, EOS, MCV, MCHC and MCH. HET was significantly ($p < 0.05$) higher in the conditioned group (41.060%) than in the unconditioned group (36.760%) and LYM was significantly ($p < 0.05$) lower in the conditioned group (55.680%) than in the unconditioned group (59.360%). Thermal conditioning significantly ($p < 0.05$) increased H/L ratio (Table 6).

Table 6
Effect of Thermal Conditioning on Haematological Parameters (Means \pm S.E)

Parameters	Conditioned	Unconditioned
PCV	31.707 \pm 1.555	28.200 \pm 1.610
HB	10.567 \pm 0.515	9.428 \pm 0.533
RBC	2.334 \pm 0.120	2.140 \pm 0.124
HET	41.060 \pm 1.077 ^a	36.760 \pm 1.115 ^b
LYM	55.680 \pm 0.992 ^b	59.360 \pm 1.027 ^a
H/LR	0.755 \pm 0.031 ^a	0.629 \pm 0.032 ^b
EOS	2.693 \pm 0.199	2.800 \pm 0.20
MCV	14.379 \pm 4.9283	13.111 \pm 0.697
MCHC	0.333 \pm 0.0041	0.335 \pm 0.0051
MCH	4.793 \pm 1.6408	4.393 \pm 0.2143
PCV: Packed Cell Volume (%), HB: Haemoglobin (g/dL), RBC: Red Blood Cell (%), HET: Heterophil (%), LYM: Lymphocytes (%), H/LR: Heterophil/Lymphocyte Ratio, EOS: Eosinophil (%), MCV: Mean Corpuscular volume, MCHC: Mean Corpuscular Haemoglobin MCH: Mean Corpuscular Haemoglobin Concentration.		

The interactive effect of strain and thermal conditioning was significant ($p < 0.05$) on all haematological parameters. Thermal Conditioning (TC) significantly ($p < 0.05$) increased PCV, HB and RBC in C500 and R308, had no effect in FA. Conditioned Cobb had significantly ($p < 0.05$) higher means of 37.200%, 12.340 g/dL and 2.780% than all the other strains in PCV, HB, RBC respectively. Unconditioned R308 reversed the order. The HLR of FA was lower in the conditioned group than in the unconditioned. EOS values varied from 2.167–3.500% with the highest means in Conditioned FA (Table 7).

Table 7
Effect of Strain by Thermal Conditioning Interaction on Haematology (Means \pm S.E)

Parameters	FA CON	FA UNC	C CON	C UNC	R CON	R UNC
PCV	33.67 \pm 2.43 ^{ab}	33.00 \pm 2.32 ^{ab}	37.20 \pm 2.48^a	25.40 \pm 2.33 ^b	25.40 \pm 2.33 ^b	26.00 \pm 2.58 ^b
HB	11.10 \pm 0.796 ^{ab}	10.98 \pm 0.832 ^{ab}	12.34 \pm 0.716^a	8.48 \pm 0.806 ^b	8.48 \pm 0.806 ^b	8.68 \pm 0.806 ^b
RBC	2.47 \pm 0.208 ^b	2.58 \pm 0.223 ^b	2.78 \pm 0.223^a	1.92 \pm 0.223 ^{bc}	1.92 \pm 0.223 ^{bc}	1.96 \pm 0.223 ^{bc}
HET	37.66 \pm 2.32 ^b	38.00 \pm 2.41^b	43.00 \pm 2.32 ^{ab}	36.20 \pm 2.34 ^{bc}	36.20 \pm 2.34 ^{bc}	35.00 \pm 2.34 ^c
LYM	58.50 \pm 1.624 ^{ab}	58.00 \pm 1.555 ^b	53.40 \pm 1.555 ^{bc}	60.00 \pm 1.624 ^a	60.00 \pm 1.624 ^a	60.60 \pm 1.624 ^a
H_L_R	0.65 \pm 0.037^{ab}	0.66 \pm 0.039 ^{ab}	0.82 \pm 0.03 ^a	0.61 \pm 0.029 ^b	0.61 \pm 0.029 ^b	0.60 \pm 0.032 ^b
EOS	3.50 \pm 0.31^a	3.00 \pm 0.23 ^{ab}	2.40 \pm 0.23 ^b	3.20 \pm 0.23 ^{ab}	3.20 \pm 0.23 ^{ab}	3.00 \pm 0.23 ^{ab}
MCV	13.69 \pm 0.335 ^b	12.6 \pm 0.335 ^b	13.39 \pm 0.335 ^b	12.99 \pm 0.345 ^b	12.99 \pm 0.345 ^b	13.14 \pm 0.433 ^b
MCHC	0.333 \pm 0.03 ^{ab}	0.329 \pm 0.02 ^b	0.332 \pm 0.11 ^{ab}	0.34 \pm 0.03 ^a	0.34 \pm 0.03 ^a	0.34 \pm 0.03 ^a
MCH	4.50 \pm 0.04 ^b	4.21 \pm 0.04 ^b	4.44 \pm 0.133 ^b	4.35 \pm 0.133 ^b	4.35 \pm 0.133 ^b	4.4 \pm 0.061 ^b
C = C500, R = R308, CON = Conditioned, UNC = Unconditioned						

BDNF gene expression

The BDNF gene was most expressed in FA and least expressed in C500 strain (Fig. 1).

Thermal conditioning significantly ($p < 0.05$) increased BDNF expression (Fig. 2).

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. 3). However, there was no statistical difference between conditioned and unconditioned FA.

Discussion

Heat stress parameters indicated that FA was less stressed than the two exotic strains. This may be attributed to genetic differences in heat tolerance. Indigenous chickens have greater potential for tropical adaptability and disease resistance than the exotic under the same environmental conditions (Ajayi, 2010; Mahendra, 2016). The reduction in heat stress parameters for conditioned chicks can be attributed to acclimation. There was a sharp increase in heat stress experienced by unconditioned chicks due to acute heat challenge which conditioned chicks were already prepared for. The ability of poultry to resist heat stress is improved if they are exposed to high environmental temperatures during the neonatal period as postulated by Collier *et al.* (undated). The interactive effect further confirms that thermal conditioning alleviated the effect of the acute heat exposure, especially for the exotic strains (C500 and R308).

Haematological parameters have been studied to understand the relationship of blood characteristics to the environment (Ovuru and Ekweozor, 2004) and have been suggested for use in the selection of animals that are genetically resistant to certain diseases and environmental conditions (Isaac *et al.*, 2013; Mmereole, 2008). Although the three strains used in the study had statistically similar blood profiles, it is only the FA strain mean values that falls within reference ranges (Onyishi *et al.*, 2017) for important erythrocytic and leukocytic indices. A higher level of PCV, Hb, RBC and EOS observed in FA strain could imply it has a healthier status since Packed Cell Volume and Hb are involved in the transport of oxygen and absorbed nutrients (Isaac *et al.* 2013; Maton *et al.*, 1993).

Effect of the Strain X Thermal Conditioning interaction on haematological parameters in this study (i.e. Genotype X Environment) varied among the strains. Thermal conditioning increased PCV, HB and RBC in the two commercial broiler strains, C500 and R308, whereas it had no effect on FA. This agrees with the findings of Siegel (1989) who reported that various lines exhibited different responses in the same environmental conditions because of the Genotype X environment interaction.

BDNF gene is essential for synaptic plasticity and maintenance of long-term memory (Barco *et al.*, 2005; Kang and Schuman, 1995); increase in its expression has been found in heat conditioned chicks when compared to unconditioned chicks (Yossifoff *et al.*, 2008). 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 (Altan *et al.*, 2003; Nielsen *et al.*, 2003). The higher expression of BDNF gene in conditioned birds agrees with the findings of Katz and Meiri, 2006; Labunskay and Meiri, 2006; Tirosh *et al.*, 2007 and Yossifoff *et al.*, 2008. Higher expression of BDNF gene confers greater developmental plasticity which could make the thermally conditioned birds more thermo-tolerant when exposed to heat stress at marketing age (Johnston *et al.*, 1999; Johnston and Rose, 2001). However, the interactive effect of strain and thermal treatment suggests that the effect of posthatch thermal treatment is strain specific since the improved indigenous strain had the highest expression of the BDNF gene and it showed no statistical difference in the conditioned and unconditioned treatment. This possibly explains why it has lower values for heat stress parameters which are indicators of thermal stress.

Conclusion

Post hatch thermal conditioning is beneficial for alleviating thermal stress in meat type chickens raised in hot climates. Although in this study, the improved indigenous strain had an edge over exotic commercial strains in terms of heat resistance, thermal conditioning enhanced thermo-tolerance across board.

Statements And Declarations

- **Ethics approval and consent to participate:** Not Applicable
- **Consent for publication:** Not Applicable
- **Availability of data and materials:** The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.
- **Declaration of Interests:** The authors declare that they have no competing interests.
- **Funding:** This research was funded by the Centre of Excellence in Agricultural Development and Sustainable Environment (CEADESE), Federal University of Agriculture, Abeokuta, Nigeria. (Grant Number: World Bank ACE 023).
- **Statement of Animal Rights:** Procedures followed for animal studies were approved by the ethics committee of the Federal University of Agriculture, Abeokuta.
- **Authors' Contributions:** Itunuola Anne Folarin acquired, analyzed and interpreted the data under the supervision of Olajide Olowofeso, Christian Obiora Ndubuisi Ikeobi, Olukayode Dewunmi Akinyemi and Olusola Thomas Oduoye as well as wrote the first draft of this manuscript. All the authors, including Babatunde Moses Ilori and Mathew Wheto, contributed to the content, as well as read, edited, and fine-tuned the manuscript. All authors approve the final manuscript.
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Figures

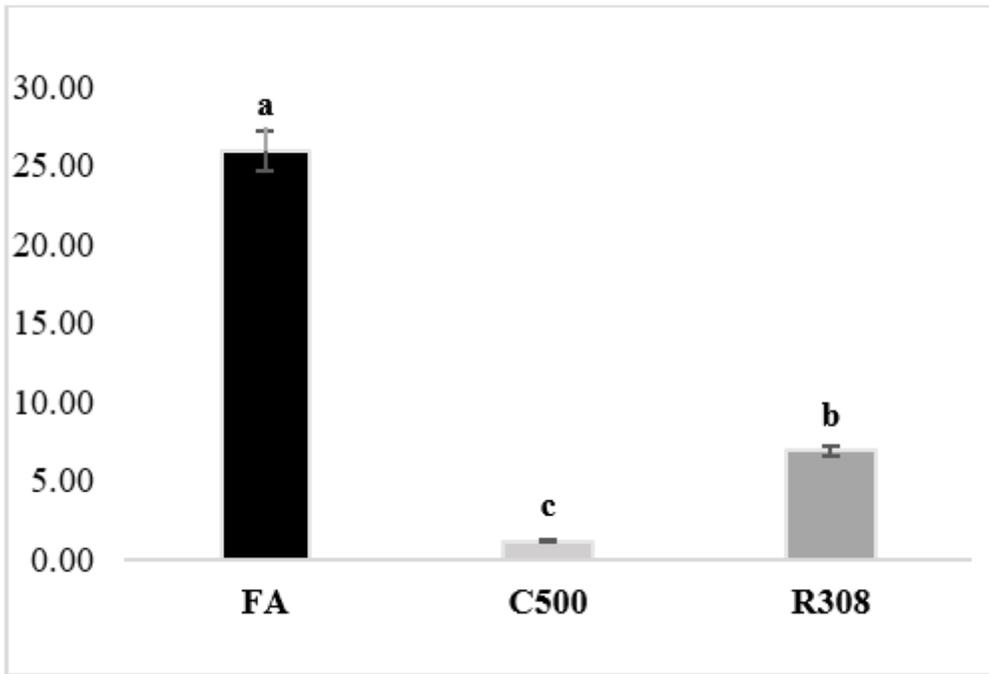


Figure 1

Effect of Strain on Relative Expression of BDNF Gene

^{a,b} Means with different superscripts are significantly different at 5% ($p < 0.05$) level.

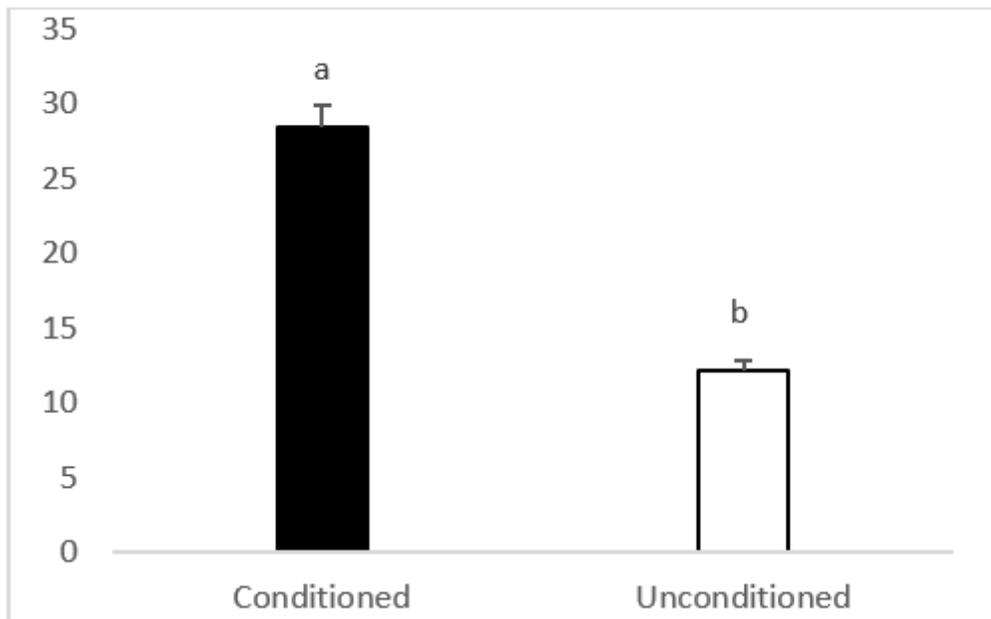


Figure 2

Effect of Thermal Conditioning on Relative Expression of BDNF Gene

^{a,b} Means with different superscripts are significantly different at 5% ($p < 0.05$) level.

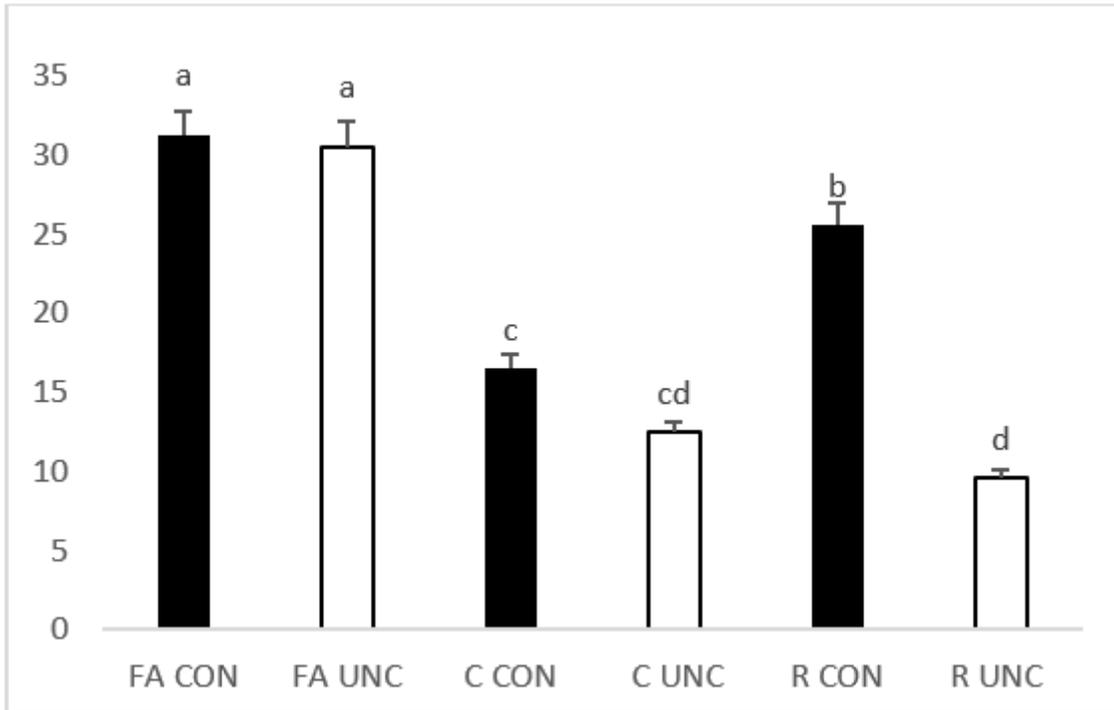


Figure 3

Effect of Strain X Thermal Conditioning Interaction on Relative Expression of BDNF Gene

^{a,b} Means with different superscripts are significantly different at 5% ($p < 0.05$) level.

C = C500, R = R308, CON = Conditioned, UNC= Unconditioned