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Thermotolerance Acquisition in Broiler Chickens through Early Feed Restriction: Response to Acute Heat Stress

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ABSTRACT

Broiler chicks were subjected to early feed restriction (EFR) on d3-5 post-hatch to determine the day that confers the best thermal tolerance during acute heat stress episode at market age. In Experiment I, 160 *Marshall* chicks were allotted to 4 treatments. One group received feed *ad libitum* (CONTROL), while others received no feed for a period of 24 hours on d3 (D3), d4 (D4) and d5 (D5), respectively. Each group had 4 replicates with a total of 10 birds per replicate. On d55 of age, rectal temperature (RT) was monitored and blood samples were taken from the birds before and 1h after exposure to temperature 37±2°C and 50% relative humidity. There were a total of 120 chicks in Experiment II and they were divided into 3 groups which were either fed *ad libitum* (CONTROL) or had feed withdrawn for 12h ($D5^{12}$) or 24h ($D5^{24}$) on d5 to determine the length of feed restriction that best conferred thermotolerance on the birds. RT and blood sampling were also done as described above. In Experiment I, haematological parameters were ($P>0.05$) similar among the treatments before and after exposure. Prior to the exposure to heat stress, RT was not affected ($P>0.05$) by feeding regimen but it was significantly ($P<0.05$) affected by the treatment after the exposure. D5 chickens had significantly ($P<0.05$) lower temperature than control. However, it was not different from D3 and D4 chickens. In Experiment II, initial RT did not differ among the 3 treatment groups although RT was ($P<0.05$) affected by the feeding regimen after 60 minutes of exposure. The control chickens had similar RT with that of $D5^{12}$. However, RT in control birds ($P<0.05$) was higher than RT in $D5^{24}$. Thus, applying EFR in broilers on D5 of post-hatch for 24

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hours may help reduce hyperthermia during heat spell at market age.

Keyword: rectal temperature, acute heat stress, broilers, H:L, early feed restriction

INTRODUCTION

Broiler production continues to suffer under the influence of heat stress in the tropics (Lin *et al.*, 2006; Abioja, 2010; Sunil Kumar *et al.*, 2011) where environmental temperature is often above thermal comfort zone of most poultry species. It results in hyperthermia (Altan *et al.*, 2000; Garriga *et al.*, 2006) which leads to a cascade of events that affect the well-being of the birds. Heat stress causes changes in leucocyte number and other haematological parameters (Maxwell *et al.*, 1992; Yalcin *et al.*, 2004).

Broilers are more susceptible at growing phase and at market age than starting phase. The envisaged increase in environmental temperature, as climate change becomes more evident, has created fear in broiler industry. Moreover, IPCC (2007) report pointed to Africa as a continent that is most likely to be affected by climate variations. Climate change, in terms of both climate means and variability, poses a great threat to farmers and the livestock in the region.

Stress is usually avoided because of its perceived repercussions on living tissues. However, evidences are emanating from various research works that exposure of broiler chicks to mild stress at early age help in acquiring thermotolerance at latter age. This was first reported in rats by Levine (1962). The author discovered that mild stress at early age affects the adrenocortical functioning during adulthood. It was later confirmed in broiler chickens (Yahav and Plavnik, 1999; Zulkifli *et al.*, 2000; Liew *et al.*, 2003). From this assertion, there are two strategies that have been explored: thermal conditioning (TC) and early feed restriction (EFR). TC involves exposing chicks at early age post-hatch to elevated ambient temperature (Arjona *et al.*, 1990; Yahav and Hurwitz, 1996; Yahav and McMurtry, 2001) within the first six days while EFR subjected the chicks to varying degrees of hunger during the same period (Zulkifli *et al.*, 1994). TC has been demonstrated to be effective as a tool for thermotolerance acquisition in poultry but its application is not practicable in the traditional open-sided poultry houses common in most tropical and developing countries. The cost of heating poultry house to a desired temperature is high. Besides, the technicality associated with thermal conditioning such as length and degree of heat exposure may not be easily mastered by the local farmers. This leaves the only option for thermotolerance acquisition in the developing and under-developed world to application of early feed restriction.

Zulkifli *et al*. (2000) reported that acute heat stress resulted in increase in heterophil/lymphocyte ratio for all feed restricted groups and the *ad libitum* group. But broilers restricted from 60% of daily feed requirement on day 4, 5 and 6 had the least heterophil/lymphocyte ratio. The authors concluded it appeared 60% feed restriction is beneficial in improving growth

and survivability of female broiler chickens exposed to heat stress later in life. The authors restricted the feed by percentage on three consecutive days (day 4, 5 and 6). Exact feed intake of the birds may not be ascertained: feed intake depends on strain, genotype, environmental condition in the pen, body weight, *etc*. Farmer may have problem calculating the feed intake. Besides, percentage feed restriction may not give the exact day the EFR is most effective. Therefore it is important to determine exact day of life the chicks should be subjected to early feed restriction by farmer which ensures best acquisition of thermotolerance.

Therefore, the objective of this study is to determine the effectiveness of EFR in conferring thermotolerance on broilers at market age with respect to change in blood parameters and rectal temperature during acute heat stress. It also sought to establish the actual day and length of time chicks should be exposed to EFR which will ensure the least fluctuation in body temperature and blood picture.

MATERIALS AND METHODS

Meteorological observations: Minimum, maximum and mean temperature, and relative humidity at the level of the birds in the pen were monitored by means of digital thermo-hygrometer.

Animals and Management

Experiment I: This focused on appropriate day of exposure to EFR that confers the best thermotolerance on broiler chicks. One hundred and sixty *Marshall* broiler chicks obtained from a reputable hatchery were allotted to four treatments. One group received feed *ad libitum* (CONTROL) while others received no feed for a period of 24 hours on day 3 (D3), day 4 (D4) and day 5 (D5) respectively. Each group had 4 replicates and 10 birds per replicate. Starter (23.05% crude protein, 11.73 MJ/Kg, 3.93% ether extract and 3.67% crude fibre) and finisher mash (19.91% crude protein, 11.71 MJ/Kg, 3.89% ether extract and 3.79% crude fibre) were supplied *ad libitum* to all birds except on days of feed restriction while water was made available always.

Acute heat stress: Four birds per treatment were transferred from the pen into a pre-heated room (37±2^{o}C, 50% RH) to test heat tolerant response to 1-hour acute heat stress episode on d55. The temperature was 24^{o}C before the chickens were moved from the pen.

Blood analyses: Blood samples were collected from the birds via brachial vein before the transfer and 1 hour after transfer into the pre-heated room. The samples were centrifuged and the plasma stored at -20^{o}C for analyses. Wintrobes microhaematocrit and colorimetry methods (Lamb, 1991) were used to determine packed cell volume (PCV), haemoglobin concentration (Hb), red blood cell count (RBC) and white blood cell count (WBC). Blood collected into labeled EDTA bottles were placed in the microhaematocrit centrifuge and spun for 5 minutes at a revolution of 1988 x g. The PCV values were subsequently determined by measuring the height of

the red cell column and expressing this as a ratio of the height of the total blood column using microhaematocrit reader. Red blood cell count was done by diluting the blood sample with 0.9% NaCl and shaking well. The diluted blood was mounted on a haemocytometer and the number of erythrocytes counted microscopically. Four bBlood smears were stained using May-Grunwald and Giemsa stains approximately 4 h after preparation with methyl alcohol fixation. Leucocyte differentials (heterophils, lymphocytes, eosinophils, monocytes and basophils) were counted for each smear and heterophil:lymphocyte ratio was calculated according to Yalcin *et al.* (2005).

Blood glucose was determined using the enzymes glucose oxidase and peroxidase (Belo *et al.,* 1976). The former catalyses the oxidation of glucose to glucoronic acid and hydrogen peroxide. The hydrogen peroxide in the presence of the second enzyme and a chromogenic hydrogen donor, d-dinisidine forms a coloured substance. The following

TABLE 1
Haematological parameters and blood glucose (before and after acute heat stress episode) of broiler chickens exposed to 24-hour feed withdrawal during d3, d4 or d5 of age

Parameter		CONTROL	D3	D4	D5	Sem	P
Packed cell volume (%)	Before	32.3	33.5	32.5	34.0	1.33	0.97
	After	31.0	33.8	33.5	34.3	0.72	0.42
Haemoglobin concentration (g/dL)	Before	8.8	9.0	9.1	10.4	0.45	0.63
	After	9.0	10.0	8.8	9.0	0.37	0.70
Red blood cell ($x10^{12}$/L)	Before	2.3	2.2	2.1	2.0	0.13	0.79
	After	1.7	2.2	1.9	1.9	0.10	0.44
White blood cell ($x10^{9}$/L)	Before	11.9	14.7	15.2	13.7	0.69	0.35
	After	12.5	12.0	12.7	14.0	0.57	0.67
Heterophil (%)	Before	27.0	36.8	34.5	33.3	1.53	0.12
	After	32.3	35.0	33.3	34.3	1.62	0.95
Lymphocyte (%)	Before	72.0	62.5	64.8	65.8	1.59	0.18
	After	66.5	64.0	66.0	64.8	1.74	0.97
Eosinophil (%)	Before	0.25	0.25	0.50	0.00	0.144	0.73
	After	0.25	0.25	0.25	0.25	0.112	1.00
Basophil (%)	Before	0.00	0.25	0.25	0.75	0.151	0.38
	After	0.50	0.50	0.25	0.25	0.125	0.83
Monocyte (%)	Before	0.75	0.00	0.25	0.75	0.144	0.22
	After	0.50	0.50	0.25	0.25	0.125	0.83
Glucose (mg/dL)	Before	149.8	148.0	132.8	146.5	3.98	0.45
	After	144.5	150.8	148.5	148.5	3.10	0.93
Heterophil:Lymphocyte ratio	Before	0.39	059	0.54	0.51	0.33	0.14
	After	0.50	0.57	0.51	0.54	0.04	0.95

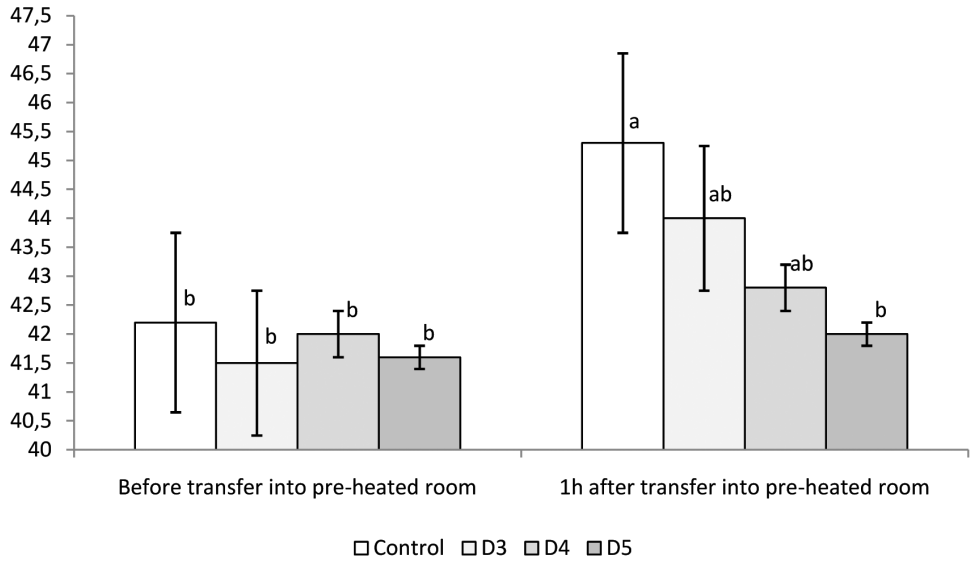
procedure was used: 1:10 somogyi-filtrate stock was prepared by adding 0.2 ml of blood to a test tube with 1 ml distilled water. After this, 0.4 ml of 0.3N barium hydroxide was added and stirred vigorously. After 5-10 minutes, 0.4 ml of 5% zinc sulphate and filtered. 0.1 ml of the somogyi-filtrate was put into a tube and mixed with 1 ml of the glucose oxidase enzyme. A blank with 0.1 ml water and standard with 0.1 ml of glucose solution was included with each set of unknowns. All the tubes were incubated in a water bath thermostatically maintained at 37^{o}C for 30-40 minutes. After incubation, 5 ml H_2SO_4 was added to each tube and mixed. The pink colour was read at 540mU against the blank tube (Spectronic® 20D model).

Rectal temperature: The rectal temperature (RT) of the birds was measured using *Jorita* digital thermometer (±0.1^{o}C accuracy, model ECT-5) inserted into the rectum (colon) of the birds and held till the thermometer beeped as described by Abioja *et al.* (2012). The readings were taken before and 1 hour after being transferred into the pre-heated room.

Experiment II: Subsequently *Experiment II* focusing on the length of EFR on day 5 was designed. One hundred and twenty day-old chicks were divided into three treatment groups of 40 each. Group I was fed *ad libitum* (CONTROL) while the remaining 2 groups had feed withdrawn for 12 hours ($D5^{12}$) or 24 hours ($D5^{24}$). The methods employed and data collection are the same to those of Experiments I. Data were subjected to analysis of variance using SYSTAT (1992) computer statistical package. Ninety five percentage level of confidence ($P<0.05$) was taken as significant. Means that are statistically different were separated with Tukey test.

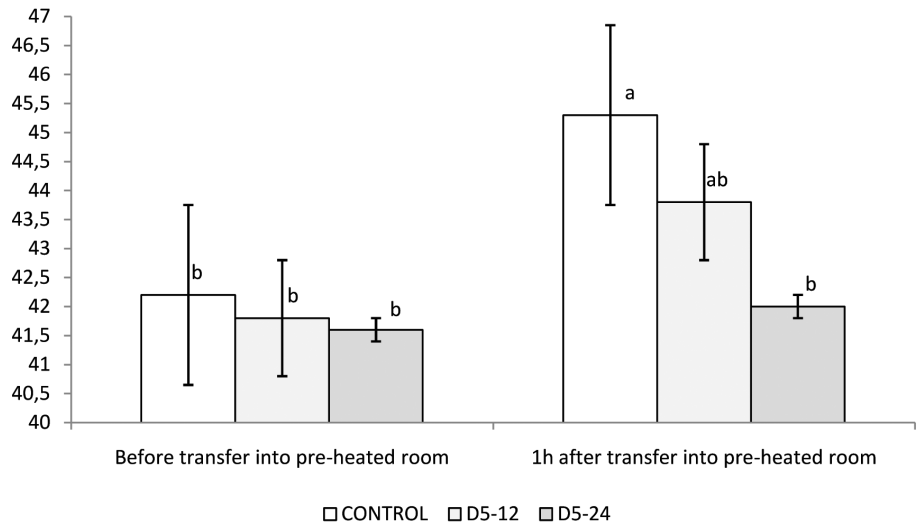
RESULTS AND DISCUSSION

Fig.1 shows the RT of the broiler chickens before and after the exposure to heat stress in Experiment I. There was no significant ($P>0.05$) difference in the RT of the birds in the four treatments before the exposure. The RT ranged between 41.5 and 42.2^{o}C. However, after 60 minutes of heat episode, RT revealed significantly ($P<0.05$) higher value in the control chicks than those exposed to EFR on D5. D3 and D4 birds had similar RT with that of the control. The change in the RT of birds in the four groups was not statistically ($P>0.05$) different (data not presented). The results of haematological parameters and blood glucose (before and after acute heat stress episode) in broiler chickens exposed to 24-hour feed withdrawal during D3, D4 and D5 of age are presented in Table 1. For packed cell volume, haemoglobin concentration, red blood cell count, white blood cell count, differential counts, H:L and blood glucose, there were no significant ($P>0.05$) differences among the four groups before and after the heat stress exposure. Fig.2 presents the results of the initial and final rectal temperature of broilers feed restricted on day 5 for either 12 or 24 hours. The initial RT was not ($P>0.05$) different from each other. Nevertheless, exposure to acute heat stress resulted in different ($P<0.05$)



[a,b] Means with different superscripts differ significantly (P<0.05)

Fig.1: Rectal temperature (before and after acute heat stress episode) of broiler chickens exposed to 24-hour feed withdrawal during d3, d4 or d5 of age



[a,b] Means with different superscripts differ significantly (P<0.05)

Fig.2: Rectal temperature (before and after acute heat stress episode) of broiler chickens exposed to 12 and 24-hour feed withdrawal during d5 of age

responses in the birds. In particular, $D5^{24}$ chickens had lower RT than that of the CONTROL chicks. The RT of the $D5^{12}$ broilers fell in between the two.

Rectal temperature is used as a major indicator of heat stress in poultry (Abioja *et al.*, 2012). Heat stress leads to elevated body temperature as the environmental temperature shoots ahead of the comfort zone of the birds (Kumar *et al.*, 2011). All the broiler chickens in this study experienced increased rectal temperature during acute heat stress. However, the increment was the highest in birds that had no prior treatment of EFR (Zulkifli *et al.*, 1994). EFR for 24 hours on day 5 gave the least increment in body temperature. This finding shows that they acquire heat tolerance that helps them in limiting the increased body temperature. Similarly, Yahav and Hurwitz (1996) reported that male broiler chickens exposed to thermal conditioning during d5 and d7 had lower body temperature when subjected to acute heat stress (35±1^{o}C; 20-30% relative humidity) than broiler that did not experience thermal conditioning. Yahav and Plavnik (1999) corroborated the report of Yahav and Hurwitz (1996). Birds on restriction on day 3 and day 4 could also tolerate the elevated ambient temperature to some extent but not as much as those on day 5. Most authors who had worked on EFR did not report on its effects on body temperature. Comparing the duration of EFR, the results showed that a whole day restriction conferred a better heat tolerance on the broiler chickens than half day restriction.

Although early feed restricted broiler chickens could resist the effects of excess environmental temperature by limiting the increase in body temperature, there was no influence of EFR in any day compared with the control in haematological parameters and blood glucose. This finding is similar to that of Zulkifli *et al*. (2000) who stated that although exposing the birds led to an increment in blood glucose, the concentrations of glucose were not affected by feeding regimes. In other words, the stress imposed by feed restriction may not be sufficient enough to elicit the thermotolerant response anticipated. Moreover, feed withdrawal was only done for a period of 24 hours. In contrast, Zulkifli *et al.* (2000) reported that 60% EFR was found to have lowered H/L ratio compared to the results of the present study where there was no difference detected in the H/L ratio regardless of whether the restriction was on day 3, day 4 or day 5.

It can be concluded that blood parameters of acutely heat-stressed broilers were not affected by EFR. However, applying EFR to broiler chicks of d5 post-hatch for 24 hours may help reduce hyperthermia that is common during heat spell in pen at market age.

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