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The Effects of the Hot, Humid Tropical Climate and Early Age Feed Restriction on Stress and Fear Responses, and Performance in Broiler Chickens

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ABSTRACT : The present study was conducted to determine the effects of two types of housing systems and early age feed restriction on stress and fear reactions, and performance in broiler chickens raised in a hot, humid tropical climate. On day 1, chicks were housed either in windowless environmentally controlled chambers (temperature was set at 32°C on day 1 and gradually reduced to 23°C by day 21) or in conventional open-sided houses (OH) with cyclic temperatures (minimum, 24°C; maximum, 34°C). An equal number of chicks from each housing system was subjected to either *ad libitum* feeding (AL) or 60% feed restriction on day 4, 5 and 6 (FR). The CH birds showed greater weight gain, higher feed consumption and better feed conversion ratios (FCR) than their OH counterparts. Feeding regimen had negligible effect on overall performance. Neither housing nor feeding regimen had a significant ($p<0.05$) effect on mortality rate. Although the CH birds were less stressed, as measured by plasma corticosterone concentration (CORT), than those of OH, the former showed longer TI duration suggesting higher magnitude of underlying fearfulness. A significant ($p<0.05$) effect of housing on heterophil/lymphocyte ratios was only noted among the AL birds where the CH birds had higher values than OH. Collectively, these results suggest that although OH birds had poorer performance and higher level of stress than CH, the former were less fearful. Although FR had negligible effect on growth performance, the regimen alleviated both stress and fear reactions in broilers. (**Key Words :** Broiler Chickens, Feed Restriction, Hot and Humid Climate)

INTRODUCTION

Tropical countries like Malaysia are constantly facing the challenge of excessive heat and humidity. The hot, humid climate can have a damaging effect on performance and well-being of poultry. Depressed growth rate and decreased feed consumption of broilers raised in higher temperature environments have been reported in many studies over a number of years (Daghir, 1995a). To optimize feed utilization and weight gain of broiler chickens, environmental temperature should be about 23°C. However, in Malaysia where the ambient temperature ranges from 24°C to 34°C, maintaining an optimum climatic environment in a conventional open-sided house is a challenge. Most of the earlier work on heat stress in poultry involved growing of chickens under artificially controlled climates. There is little information on the effects of the natural cyclic temperatures with a maximum of 34°C on the performance and physiology of broiler chickens as compared to those grown under thermoneutral conditions.

Previous studies (Zulkifli et al., 1994a, b, 2000a) under artificially controlled conditions have consistently shown that early age feed restriction enhanced the ability of chickens to withstand high ambient temperatures as juveniles than those fed *ad libitum* throughout the experiment. The only reported study on early age feed restriction and heat tolerance in chickens under the natural tropical hot, humid conditions is by Zulkifli et al. (2004). The authors reported that the early age feed restricted birds had better cumulative feed conversion ratios, and survivability rate than those fed *ad libitum* throughout.

Work carried out with both meat- and egg-type chickens suggest that stress responses attributable to beak trimming (Lee and Craig, 1991), transportation (Zulkifli et al., 2000), forced molting (Campo and Carnicer, 1994) and social

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disruption (Jones and Williams, 1992) may augment fearfulness. Literature concerning the influence of thermal stress on underlying fearfulness in chickens is conflicting. Campo and Carnicer (1994) reported that heat-stressed laying hens were less fearful than controls, as measured by tonic immobility (TI) duration. On the contrary, Zulkifli et al. (1999) noted negligible effect of heat stress on TI duration in both commercial broiler chickens and the red jungle fowl. Present study was undertaken because there is almost no scientific information on stress and fear responses of broilers raised in a conventional open-sided house under the hot, humid tropical climate as compared to those reared in thermoneutral conditions. In this study, HLR and CORT were used as indices of stress. There are numerous reports that TI is a robust measure of bird's fearfulness and is positively correlated to the antecedent fear state and it is considered a particularly useful and reliable index of general, underlying fearfulness in poultry (Jones, 1986).

MATERIALS AND METHODS

Birds, husbandry and experimental procedure

A total 432 day-old female commercial broiler chicks (Cobb) were obtained from a local hatchery. On day 1, the birds were individually weighed and wing-banded. Two hundred and sixteen chicks were placed at random in groups of six to 36 cages in three-tiered batteries with wire floors in three windowless environmentally controlled chambers (12 cages per chamber) (2.3×9.1×3.8 m) (CH). Floor space allowed was 923 cm^2 per bird. Ambient temperature on day 1 was set at 32°C and gradually reduced to 23°C by day 21. The relative humidity was between 65 to 75%. The remaining 216 chicks were placed in similar battery cages and housed in three conventional open-sided houses (12 cages per house) (OH) with cyclic temperatures (minimum, 24°C; maximum, 34°C). The relative humidity was between 80 to 90%. Equal number of chicks for each housing system was subjected to either *ad libitum* feeding (AL) or 60% feed restriction on day 4, 5 and 6 (FR). Food restriction was 60% of food consumption of the *ad libitum* fed group on the previous day. All birds were fed a standard broiler starter crumble (2,950 kcal ME/kg; 21% crude protein) finisher pellet (3,050 kcal; 19% crude protein) diets from age 1 to 21, and day 22 to 42, respectively. Water was available at all times. The chicks were under continuous lighting. Chicks were vaccinated against Newcastle disease via intraocular route on day 7 and 21.

Growth performance

Individual body weight and feed consumption were determined weekly. Cumulative feed conversion ratio was calculated as g food ingested per g live body weight. Mortality was recorded as it occurred.

Tonic immobility test

On day 42, 15 birds from each housing system-feeding regimen subgroup were gently caught individually with both hands, held in an inverted manner and carried to a separate room (no visual contact with other birds) for TI measurements. A modification of the procedure described by Benoff and Siegel (1976) was used. TI was induced as soon as the bird arrived in the separate room by gently restraining it on its right side and wings for 15 sec. The experimenter then retreated approximately 1 m and remained of within the sight of the bird but made no unnecessary noise or movement. Direct eye contact between the observer and the bird was avoided as it may prolong TI duration (Jones, 1986). A stopwatch was started to record latencies until the bird righted itself. If righted in less than 10 sec, the restraining procedure was repeated. If TI is not induced after three attempts the duration of TI was considered 0 sec. The maximum duration of TI allowed was 600 sec (Zulkifli et al., 2000b). The number of inductions required to attain TI were recorded. Each bird was caught and sampled, one immediately after another. It was assumed that the catching and returning of birds did not disturb the other members of the flock (Harvey et al., 1980; Lagadic et al., 1990).

Blood sampling

Ten birds (those that were not tested for TI) from each housing system-feeding regimen subgroup were chosen at random and blood samples (0.3 ml) were obtained with EDTA as the anticoagulant from the brachial vein for the measurement of CORT and number of H and L. Individual bird was caught and sampled, one immediately after another. Time elapsed from catching to obtaining the blood sample was less than 50 s. This procedure should not influence circulating levels of CORT (Lagadic et al., 1990). Samples for CORT assay were centrifuged and stored at -20°C until assayed, using a sensitive and highly specific radioimmunoassay kit (M P Biomedical, Irvine, CA). Blood smears were prepared using May-Grunwald-Giemsa stain and H and L were counted to a total of 60 cells (Gross and Siegel, 1983).

Statistical analyses

Data were analyzed statically using General Linear Model (GLM) procedure of SAS® (SAS institute Inc., 1991). The body weight, feed intake, FCR, TI duration, and number of attempts to induce TI, HLR and CORT data were analysed using a two-way analysis of variance (SAS Institute Inc., 1991) with housing systems and feeding regimes and the interactions between them as main effects. When interactions were significant, separate ANOVA were

Table 1. Mean (±SEM) body weights, feed consumption, and feed conversion ratios (FCR) of broiler chickens by housing system and feeding regimen

	Housing system		Feeding regimen	
	OH	CH	AL	FR
Body weight (g)				
day 0	47±0.24	47±0.24	47±0.26	48±0.23
day 7	165[a]±4.30	160[b]±4.66	189[a]±1.04	138[b]±1.30
day 14	428±4.35	432±4.19	447[a]±3.21	415[b]±3.23
day 21	750[b]±19.82	801[a]±9.58	801[a]±17.15	753[b]±14.17
day 28	1218[b]±11.06	1308[a]±9.71	1287[a]±13.56	1243[b]±11.65
day 35	1604[b]±12.72	1772[a]±11.06	1708[a]±19.36	1673[b]±17.27
day 42	2141[b]±14.05	2308[a]±14.91	2,243±21.38	2,210±19.38
Feed consumption (g/bird/d)				
wk 1	153[a]±5.53	120[b]±5.58	167[a]±3.83	108[b]±3.41
wk 2	391[b]±4.29	409[a]±4.32	400±4.36	401±4.74
wk 3	539±9.50	550±12.13	549±9.12	541±12.42
wk 4	742[b]±7.42	813[a]±8.30	784±12.32	772±8 .22
wk 5	820[b]±14.15	882[a]±7.25	846±14.53	857±12.20
wk 6	1,043[b]±12.68	1,087[a]±13.87	1,070±36.11	1,063±13.15
Total	3,688±30.15	3,862±32.43	3,815±36.45	3,741±33.43
Cumulative FCR (feed/gain)				
wk 1	0.92[a]±0.02	0.74[b]±0.02	0.88±0.02 [a]	0.78±0.02 [b]
wk 2	1.27[a]±0.01	1.23[b]±0.01	1.26±0.01 [a]	1.22±0.01 [b]
wk 3	1.47[a]±0.03	1.35[b]±0.01	1.41±0.03	1.41±0.03
wk 4	1.50[a]±0.01	1.45[b]±0.01	1.48±0.01	1.47±0.01
wk 5	1.65[a]±0.01	1.57[b]±0.01	1.61±0.01	1.60±0.01
wk 6	1.72[a]±0.01	1.67[b]±0.01	1.70±0.01	1.69±0.01

[a, b] Means within a column with no common letters differ at p<0.05.
OH = Conventional open-sided house with cyclic temperatures; CH: Environmentally controlled chamber.
AL = *ad libitum* feeding; FR = 60% feed restriction on days 4, 5, and 6.

conducted within each main effect. Prior to analysis, body weight and TI duration were transformed to common logarithms. CORT data were transformed to square roots. When interactions between main effects were significant, comparisons were made within each experimental variable. Untransformed means are presented in Tables. Data on mortality rate were analysed by chi-square test. All statements of significance were based on p<0.05.

RESULTS

Data on body weight, feed consumption, FCR and mortality rate are presented in Table 1. Subjecting birds to FR resulted in a significant (p<0.05) depression of BW from day 7 to day 35. However, because of accelerated growth, the body weights of the FR birds did not differ from those of AL by day 42. From day 8 to 42, feed consumption was not affected by feeding regimen. Feed conversion ratios of FR birds were significantly better than their AL counterparts from day 1 to 14. Irrespective of feeding regimen, the significant effect housing system on body weight was found on day 7, 21, 28, 35 and 42. The CH birds had significantly (p<0.05) greater body weights than their OH counterparts. CH birds consumed significantly (p<0.05) more feed and had significantly (p<0.05) better FCR than their OH counterparts. Based on chi-square analysis, neither housing nor feeding regimen had significant (p<0.05) effect on mortality rate throughout the experimental period (OH-AL, 3.7%; OH-FR, 1.8%; CH-AL, 3.7%; CH-FR, 4.5%).

There was a significant (p<0.05) housing system× feeding regimen interaction for HLR (Table 2). The interaction was observed because significant effect of housing system was only noted for those fed AL where the CH birds exhibited a significantly (p<0.05) greater increase in HLR than those of OH.

The effect of housing system and feeding regimen on

Table 2. Mean (±SEM) heterophil/lymphocyte ratios (HLR) in broiler chickens where feeding regimen by housing system interactions were significant

Feeding regimen	Housing system	
	OH	CH
AL	0.40[b]±0.05	0.61[a]±0.09
FR	0.47±0.05	0.49±0.06

[a, b] Means within a row with no common superscripts differ at p<0.05.
OH = Conventional open-sided house with cyclic temperatures; CH = Environmentally controlled chamber.
AL = *ad libitum* feeding; FR = 60% feed restriction on days 4, 5, and 6.

Table 3. Mean (±SEM) plasma corticosterone concentrations and tonic immobility durations of broilers chickens by housing system and feeding regimen

	Plasma corticosterone (ng/ml)	Tonic immobility duration (s)
Housing system		
OH	3.52±0.24[a]	175±21.29[b]
CH	2.76±0.18[b]	249±23.36[a]
Feeding regimen		
AL	3.39±0.21[a]	334.1±22.83[a]
FR	2.86±0.23[b]	240±21.66[b]

[a, b] Means within a row with no common superscripts differ at $p<0.05$.
OH = Conventional open-sided house with cyclic temperatures; CH = Environmentally controlled chamber.
AL = *ad libitum* feeding; FR = 60% feed restriction on days 4, 5, and 6.

plasma levels of CORT is shown in Table 3. There was no significant ($p>0.05$) housing system×feeding regimen interaction for plasma CORT. Irrespective of feeding regimen, CORT was significantly ($p<0.05$) affected by housing system. The OH birds had higher CORT than those of CH. Subjecting birds to AL resulted in a higher CORT compared to FR. There was no significant ($p>0.05$) housing system×feeding regimen interaction for TI duration Irrespective of feeding regimen, CH birds had longer TI duration than those of OH (Table 3). FR significantly ($p<0.05$) reduced TI duration as compared to those of AL.

DISCUSSION

The effects of OH, where the birds were exposed to the hot, humid tropical climate, on body weight, feed intake and FCR are in agreement with previous studies (Ain Baziz et al., 1996; Geraert et al., 1996; Zulkifli, 1999; Zulkifli et al., 2004; Abu-Dieyeh, 2006). Modern fast growing broiler chicks must consume sufficient quantity of feed in order to attain maximal growth rate. However, the intake and metabolism of feed have a thermogenic effects and at high ambient temperatures this heat increment exacerbates the problem by adding more heat to an already heat distressed birds. The bird, thus, reacts by lowering its voluntary feed intake and its metabolic rate. This results in poorer FCR and body growth. Zuprizal et al. (1993) and Siegel (1995) attributed the reduction in body weight gain during heat stress episode to the reduction in both feed consumption and true digestibility of protein and amino acids.

As expected, the FR retarded the growth rate of birds. The mean body weight of FR birds on day 7 was approximately 73% of those AL. The present study confirms previous observations that when birds were returned to *ad libitum* feeding, compensatory growth took place and they grew rapidly to catch up with AL birds (Zulkifli et al., 1994a; b; 2000a) Zulkifli et al. (2004) reported that degree of compensatory growth following release to *ad libitum* feeding was strain dependent with Hubbard×Hubbard chickens exhibited a greater rate of growth than those of Cobb×Cobb. Results of this experiment concur with those of Zulkifli et al. (2004) that Cobb×Cobb birds attained full compensatory growth following FR at 42 days of age. The lack of significant house type×feeding regimen interaction suggested that the degree of compensatory growth following release was not dependent on ambient temperature. Zulkifli et al. (1994; 2000a) presented evidence that FR can improve the body weight of chickens exposed to 38°C for 2 h/d form day 35 to 41. On the contrary, the present findings and those of Zulkifli et al. (2004) suggested that FR had negligible effect on the market age body weights (day 42) of broilers under the hot, humid tropical climate. Because the birds were exposed to different thermal conditions, inferences should be made with caution. In practice, growing chickens are subjected to a wide range of diurnally cycling temperatures rather than a constant one. According to Yahav et al. (1996) the growth rate of 4- to 8-week-old chickens exposed to cyclic temperatures of 15:30°C was similar to that of others exposed to a constant temperature corresponding to the average. Hurwitz and Bengal (1982) found that the performance of turkeys kept in diurnally cycling temperatures of up to 30°C was similar to that achieved by others exposed to the corresponding constant temperature. However, when the upper temperature exceeded 30°C, the growth of turkeys exposed to cyclic temperatures was inferior to that obtained at the upper temperature. Although Zulkifli et al. (2004) noted significant improvement in the FCR of birds subjected to early age fasting and raised under the hot, humid tropical conditions, the present study indicated that both of FR and AL birds had similar FCR. There is no clear explanation for the discrepancies.

House type had no significant effect on mortality rate. Thus, it appears that although the heat stress experienced by the birds raised in OH was detrimental to their growth performance, they were able to survive the hot, humid tropical environment. Early age fasting has also been shown to reduce the mortality rate of birds subjected to the hot, humid tropical environment (Zulkifli et al., 2004), and a constant temperature of 38°C for 2 h/d from day 35 to 41 (Zulkifli et al., 2000a). However, in the present study, the mortality rates of the FR and AL birds raised in OH were not significantly different. There is possibility that the AL birds raised in OH have experienced a certain degree of acclimatization following the continuous exposure to the tropical environment commencing from the neonatal age (Arjona et al., 1988; Yahav and McMurtry, 2001). Earlier studies (Daghir, 1995b) demonstrated the possibility of acclimatizing chickens to hot environment by daily exposures to elevated temperatures. Heat acclimatization elicits adaptations that regulate dehydration and hypovolaemia (Van Kampen, 1981). These adaptations tend to maximize body water reserves needed for evaporative cooling and blood volume maintenance.

The present findings, as measured by CORT on day 42, clearly demonstrated that raising birds under OH was more stressful than CH. Heat stress stimulates the release of corticosterone from the adrenal glands and increased plasma concentrations of corticosterone in chickens (Zulkifli et al., 2009) The high relative humidity of the OH environment may have heightened the heat distress experienced by the chickens. It is well established that at high ambient temperature, heat dissipation by evaporative cooling is impeded by high relative humidity. Hence, it appears that the OH birds failed to fully acclimatize to the hot, humid tropical environment despite the continuous exposure to the environment commencing from the neonatal age.

The lower CORT in the FR birds as compared to those of AL confirm earlier studies on the benefit of early age feed restriction (Zulkifli et al., 1994a, b; 1995; 2000a; Liew et al., 2003). It has been shown that acquired enhanced heat tolerance resulting from FR could be attributed to improved heat shock protein (hsp) 70 response (Liew et al., 2003). The hsp play a profound role in regulating protein folding and in coping with proteins affected by heat and other stressors (Gething and Sanbrook, 1992).

Increases in HLR have been reported to be a more reliable indicator of chronic stress than CORT (Gross and Siegel, 1983; Maxwell, 1993). It is interesting to note that among the AL birds, despite being raised in a hotter and more humid environment, the OH chicks had lower HLR than their CH suggesting the latter was more distressed. The phenomenon could be associated with the more rapid growth rate attained by the CH birds. Al-Murrani et al. (1997) showed that light-bodied Iraqi fowl had significantly lower HLR than the heavy Iraqi meat-type line. Similarly, larger turkeys have been reported to show lower L and higher H counts than did the smaller birds (Bayyari et al., 1997; Huff et al., 2005).

There are numerous reports that TI is a robust measure of bird's fearfulness (Jones, 1986). The positive relationship between antecedent fear state and the duration of TI has been extensively reviewed (Jones, 1986). Fear is an important component of stress and prolonged or intense fear can markedly reduce welfare and performance. Based on TI duration, the CH birds were more fearful than their OH counterparts. Literature regarding the influence of heat stress on underlying fearfulness is conflicting. Campo and Carnicer (1994) showed that heat exposure augmented TI reaction. On the contrary, working with the commercial broiler chickens and red jungle fowl, Zulkifli et al. (1999) failed to demonstrate the effect of ambient temperature on TI duration. Another possible explanation is the environmental enrichment which provides extra stimulation in home environment, that may affect birds' expectations about environmental complexity and enhance their ability to adapt to novelty. The results of present study support the theory that raising birds in OH which exposure to a wide variety of stimuli such as human activity outside their pen, which could be regarded as a form of environmental enrichment which reduces fear. This study shows that birds exposed to a wide variety of stimuli (viewing the outside area and hearing the surrounding noises), could be considered as a form of environmental enrichment, may alleviate the stress and fear reactions following catching, crating and transportation.

The shorter TI duration in FR birds as compared to those of AL was unexpected. Early age feed restriction has been shown to alleviate physiological stress responses in broiler chickens following road transportation but not underlying fearfulness (Zulkifli, 2003). Because the study by Zulkifli et al. (2003) involved road transportation which can result in extreme stress and fear reactions (Mitchell and Kettlewell, 1998), inferences should be made with caution. Although both fear and stress reactions are not synonymous, they are closely related and the explanation for the conflicting findings is equivocal.

In conclusion, under the conditions of these experiments, raising birds in conventional open-sided houses under the hot, humid tropical environment depressed growth, feed intake and FCR of broiler chickens. Birds in CH were more fearful than those of OH and this could be attributed to lack of environmental stimulation in the former. The FR had negligible effect on growth performance but alleviated both stress and fear reactions in broilers.

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