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# Effects of High Ambient Temperature on Blood Parameters in Red Jungle Fowl, Village Fowl and Broiler Chickens

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**Abstract:** Two experiments were conducted to compare heat tolerance of Red Jungle Fowl (RJF), Village Fowl (VF) and Commercial Broilers (CB) at a common age and a common body weight. In exp. 1, RJF, VF and CB of a common age (30 days old) were exposed to 36±1°C for 3 h. Creatine kinase activity was significantly higher in CB than those of RJF and VF. Both RJF and VF had significantly lower serum K and Na concentration than their CB counterparts. In exp. 2, RJF, VF and CB of common body weight (930±30 g) were subjected to similar procedures as in exp. 1. Neither genotype nor stage of heat treatment had significant effect on serum levels of cholesterol, Cl, CK and LDH. The CB was significantly more hyperglycemic than RJF following heat treatment. In both experiments, irrespective of stage of heat treatment RJF had lower heterophil/lymphocyte ratio than VF and CB. It can be concluded that intense selection for rapid growth in CB has resulted in tremendous alterations in their ability to withstand high ambient temperature as compared to the RJF and VF. It is also apparent that genetic differences in body size per sec may not determine breed or strain variations in response to heat stress.

**Key words:** Heat stress, jungle fowl, village fowl, broiler, blood parameters, Malaysia

## INTRODUCTION

The detrimental effects of heat stress on poultry production and well-being have been extensively reviewed (Howlider and Rose, 1987; Yahav, 2000; Lin *et al*., 2006). There is a wealth of literature suggesting breed and strain differences in tolerance to heat stress in chickens (Yahav *et al*., 1998; Deeb and Cahaner, 1999; Cahaner *et al*., 2008; Islam and Nishibori, 2009). Reports indicate that fast-growing broilers are more sensitive to high ambient temperature than slow-growing strain (Yunis and Cahaner, 1999). Intense selection for rapid growth in meat type chickens results in a concomitant increase in metabolic resting heat production, while heat dissipation capacity is not affected (Sandercock *et al*., 1995).

It is generally considered that native or indigenous breeds of chickens in the tropical countries are better able to withstand high ambient temperatures (Horst, 1989). At first sight it appears that Red Jungle Fowl (RJF), the ancestors of the domestic fowl, which inhabit the warmest and humid parts of Asia are highly adaptable to hot and humid conditions. Zulkifli *et al*. (1999) compared physiological responses to heat exposure in RJF and Commercial Broiler chickens (CB) at a common age and at a common body weight. When comparison was made at a common age (with large disparity in body weight), the RJF had lower rises in heterophil/lymphocyte ratios and body temperature than CB. However, comparison at a common body weight revealed no superiority among RJF over CB in the ability to withstand high ambient temperature. The researchers concluded that considerable attention should be given to genetic differences in body weight in studies of a breed effect on heat stress in poultry. Sandercock *et al*. (2006) also showed the same negative relationship between body size and heat tolerance when broilers were compared with layers. In the present study, we compared the effect of heat stress on RJF, indigenous Village Fowl (VF) and CB at a common body weight and a common chronological age.

The indigenous village fowl were the descendant of the South-East Asia red jungle fowl (*Gallus Gallus bankiva*) that have been domesticated in villages in Malaysia through natural mating and selection over a long period of time (Ramlah, 1996). Information about heat tolerance in VF is lacking. Indices used to measure the stress responses were blood biochemistry and heterophil to lymphocyte ratios. Evidences suggest that blood biochemistry such as serum concentrations of ions, protein and enzymes may be considered useful stress response measurements (Kutlu and Forbes, 1993; Lin *et al*., 2000).

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## MATERIALS AND METHODS

**Animal welfare:** The study was undertaken following the guidelines of the Research Policy of the University Putra Malaysia on animal ethics.

**Birds and husbandry:** A total of 40 female Commercial Broiler chickens (CB) (Cobb 500), 40 female Village Fowl (VF) and 40 female Red Jungle Fowl (RJF) were used in the study. The day-old CB and VF were obtained from a local commercial hatchery and Institute for Poultry and Livestock Development (Johor Baharu, Malaysia), respectively.

The RJF breeding stock was originally captured from the secondary forest and oil palm plantations in peninsular Malaysia and was assumed to be genetically pure. Purity of the RJF was assessed by gross characteristics, namely, shape and size and thickness of the bird, color of the plumage, color of the shank and ear lobes, pattern of arrangement of tail feathers and size and thickness of the comb (Vidyadaran, 1987).

The flock had maintained as a closed flock at Universiti Putra Malaysia for the last 10 years. Birds were reared in groups of 5 in three-tiered battery cages with wire floors.

Floor space allowed was 1107 $cm^2$ per bird. The batteries were in a conventional open-sided house with cyclic temperature (minimum, 25°C; maximum, 33°C). Relative humidity was between 60 and 85%. Chicks were fed commercial broiler starter (crumble form; 22% CP and 3000 kcal ME $kg^{-1}$) and finisher (crumble form; 20% CP and 3200 kcal ME $kg^{-1}$) from 1-30 days and day 31 onwards, respectively. Feed and water were available at all times and the birds were provided 12 h natural lighting.

**Experiment 1:** A total of 20 birds per genotype were used in the experiment. At 30 days of age (08:30 h; ambient temperature, 26°C), 10 birds from each genotype (mean body weights: RJF, 153±12 g; VF, 345±16 g; CB, 1.411±36 g) were randomly chosen and blood samples (3 mL) were obtained via the wing vein for determination of Heterophil to Lymphocyte Ratio (HLR), serum levels of glucose, cholesterol, total protein, Creatine Kinase (CK), sodium (Na), potassium (K) and Chloride (Cl) and Lactate Dehydrogenase (LDH).

The remaining birds (10 birds per genotype) were crated (10 birds per crate), transferred to an environmentally controlled chamber and heat stressed at 36±1°C for 3 h. Neither feed nor water was provided during the heat treatment. Immediately after heat treatment, blood samples (3 mL) were collected from each bird via the wing vein. Blood samples for HLR were transferred to tubes containing EDTA as anticoagulant. Blood smears were prepared using Wright stain and heterophil and lymphocyte were counted to a total of 60 cells (Gross and Siegel, 1983). The HLR is considered to be a reliable stress indicator in chickens (Gross and Siegel, 1983; Maxwell, 1993). Blood samples were serum separated and stored at -20°C. Analyses for serum levels of glucose, cholesterol, total protein, CK, Na, K, Cl and LDH were conducted on an automated spectrophotometer (Ultraspec® 300, Cobas-Mira, Roche Diagnostic System, CH4070 Basel, Switzerland) and using standard diagnostic kits (Roche).

**Experiment 2:** A total of 20 birds from each genotype (CB, 22 days of age; VF, 90 days of age; RJF, 150 day of age) of a common body weight (930±30 g) were subjected to heat treatment and blood sampling as described in experiment 1.

**Statistical analysis:** Data were analyzed using the GLM procedure of SAS statistical software. Data were subjected to 2-way ANOVA with the genotype and stage of heat treatment as main effects. When interactions were significant, separate ANOVA were conducted within each main effect. All statements of significance were based on $p<0.05$.

## RESULTS AND DISCUSSION

**Experiment 1:** Results for blood parameters and HLR are shown in Table 1. There were significant genotype x stage of heat treatment interactions for HLR and Cl concentrations (Table 2).

Heat treatment significantly elevated the HLR of CB but not the other two genotypes. While heat exposure had negligible effect on the serum levels of Cl of RJF and CB, the ion concentration was significantly reduced in VF. The Cl values attained in CB were consistently higher than those of RJF and VF. Irrespective of het treatment both RJF and VF had significantly lower serum CK, K, Na and glucose concentrations than their CB counterparts.

Serum CK and Na concentrations were not significantly affected by heat treatment. On the contrary, heat exposure significantly decreased serum levels of glucose and K. VF showed lower LDH concentration than their RJF and CB counterparts. Stage of heat treatment had no significant effect on LDH activity. Neither genotype nor stage of heat treatment had significant effect on serum levels of cholesterol and total protein.

**Experiment 2:** The effects of genotype and stage of heat treatment on blood parameters and HLR are shown in

Table 1: Effect of genotype and stage of heat treatment on serum levels of cholesterol, Creatine Kinase (CK), glucose, total protein, sodium (Na), potassium (K), Chloride (Cl), Lactate Dehydrogenase (LDH) and Heterophil/Lymphocyte ratio (HLR) at a common age (30 days)

| Items | Cholesterol (mmol $L^{-1}$) | CK (U $L^{-1}$) | Glucose (mmol $L^{-1}$) | Total protein (g $L^{-1}$) | Na (mmol $L^{-1}$) | K (mmol $L^{-1}$) | Cl (mmol $L^{-1}$) | LDH (U $L^{-1}$) | HLR |
|---|---|---|---|---|---|---|---|---|---|
| **Genotype** | | | | | | | | | |
| RJF† | 3.83 | 4384.00$^{b}$ | 13.340$^{b}$ | 32.540 | 144.6000$^{c}$ | 5.06$^{b}$ | 99.6900$^{c}$ | 1410.00$^{a}$ | 0.321$^{b}$ |
| VF | 3.56 | 3522.00$^{b}$ | 12.620$^{b}$ | 32.870 | 147.2000$^{b}$ | 5.12$^{b}$ | 102.8200$^{b}$ | 1224.00$^{b}$ | 0.799$^{a}$ |
| CB | 3.32 | 5739.00$^{a}$ | 14.970$^{a}$ | 30.880 | 150.8100$^{a}$ | 6.93$^{a}$ | 109.7800$^{a}$ | 1464.00$^{a}$ | 1.038$^{a}$ |
| **Stage of heat treatment** | | | | | | | | | |
| No heat | 3.53 | 4622.00 | 14.060$^{a}$ | 32.540 | 148.0900 | 6.05$^{a}$ | 104.2900 | 1414.00 | 0.601$^{b}$ |
| 3 h heat at 36°C | 3.60 | 4442.00 | 13.180$^{b}$ | 31.620 | 147.0500 | 5.35$^{b}$ | 104.0100 | 1310.00 | 0.871$^{a}$ |
| SEM | 0.08 | 241.28 | 0.240 | 0.490 | 0.5100 | 0.16 | 0.6600 | 30.82 | 0.064 |
| **Probabilities** | | | | | | | | | |
| ANOVA | | | | | | | | | |
| Genotype | NS | ** | ** | NS | ** | ** | ** | ** | ** |
| Stage of heat treatment | NS | NS | * | NS | NS | ** | NS | NS | ** |
| Genotype x stage of heat treatment | NS | NS | NS | NS | NS | NS | * | NS | * |

†RJF: Red jungle fowl, VF: Village fowl, CB: Commercial broiler; NS-Not significant; *p<0.05; **p<0.01; $^{a,b,c}$Means with no common superscripts within a column-subgroup differ significantly

Table 2: Mean (±SEM) serum Chloride (Cl) and Heterophil to Lymphocyte Ratio (HLR) where genotype x stage of heat treatment interactions were significant at common age (30 days)

| Results | RJF† | VF | CB |
|---|---|---|---|
| **Cl (mmol $L^{-1}$)** | | | |
| No heat | 98.63±0.99$^{c}$ | 104.14±0.74$^{bx}$ | 109±0.93$^{a}$ |
| 3 h heat at 36°C | 100.75±0.92$^{b}$ | 101.49±0.79$^{by}$ | 110±0.62$^{a}$ |
| **HLR** | | | |
| No heat | 0.311±0.05$^{b}$ | 0.770±0.14$^{a}$ | 0.704±0.06$^{ya}$ |
| 3 h heat at 36°C | 0.331±0.04$^{c}$ | 0.832±0.05$^{b}$ | 1.339±0.18$^{xa}$ |

†RJF: Red Jungle Fowl, VF: Village Fowl, CB: Commercial Broiler; $^{a,b,c}$Means±SEM within a row with no common letters differ at p<0.05; $^{x,y}$Means±SEM within a column with no common letters differ at p<0.05

Table 3. Significant genotype x stage of heat treatment interactions were noted for CK, glucose and LDH (Table 4). A significant effect of genotype on serum level of CK was only noted prior to heat treatment with CB exhibited the lowest level. CB but not other genotypes was significantly more hyperglycemic following heat treatment. Although, prior to heat treatment genotype had no significant effect on LDH activity, the heat treatment resulted in higher serum LDH concentration in CB and VF compared to RJF. Stage of heat treatment had no significant effect on the enzyme activity except among RJF. The serum total protein concentration of RJF was significantly higher than other genotypes throughout the duration of study. Heat treatment had negligible effect on serum levels of total protein.

Significant effect of genotype on serum level of Na was noted with a lower concentration in VF compared to other genotypes. Genotype had no significant effect on serum levels of K. Irrespective of genotype, heat treatment significantly reduced Na and K concentrations. The HLR of RJF was significantly lower than those VF and CB. Heat challenge significantly elevated HLR. The findings of experiments 1 and 2 confirmed earlier reports that heat treatment (Zulkifli *et al*., 1999, 2003, 2009; Mahmoud *et al*., 2004) may elevate HLR in chickens. Comparison at a common body weight and a common age suggested that RJF as measured by HLR was less distress than CB following heat exposure. The greater HLR in CB as opposed to RJF may also attribute to the physiological demand for rapid growth in the former (Zulkifli *et al*., 1999).

Based on the results of both experiments 1 and 2, neither genotype nor stage of heat treatment had a significant influence on serum cholesterol concentration. The effect of heat stress on serum cholesterol in chickens has been inconsistent. Kutlu and Forbes (1993) reported that exposure to high ambient temperature may result in hypercholesterolemia, while Zulkifli *et al*. (1999, 2000) noted otherwise. Alnaimy *et al*. (1992) indicated that the phenomenon could be attributed to an increase in total body water or a decrease in acetate concentration, which is the primary precursor for the synthesis of cholesterol.

Rahayu *et al*. (2008) reported that the cholesterol content of the breast and leg muscles of CB was significantly higher than RJF when compared at a common body weight (800 g). The question is whether serum cholesterol is directly related to tissue cholesterol. Studies in cattle showed that there was no linear relationship between serum and tissue cholesterol concentrations (Wheeler *et al*., 1987).

In experiment 2 as measured by CK activity, RJF and VF appeared to have more damages in their muscle than CB, which could be attributed to their higher physical activity (Tarrant and Grandin, 2000; Branciari *et al*., 2009) or older age (Hocking *et al*., 1998). It is well documented that elevated levels of CK is a useful indicator of an increase in physical activity (Tarrant and Grandin, 2000). However, CB showed higher CK activity irrespective of stage of heat treatment in experiment 1. The elevated serum level of CK in CB may be attributed to their larger body size. Sandercock *et al*. (2006) showed that at matched ages broilers exhibited more extensive idiopathic myopathy (CK activity) than layers. It has been proposed that this phenomenon is a consequence of selection for

Table 3: Effect of genotype and stage of heat treatment on serum levels of cholesterol, Creatine Kinase (CK), glucose, total protein, sodium (Na), potassium (K), Chloride (Cl), Lactate Dehydrogenase (LDH) and Heterophil/Lymphocyte Ratio (HLR) at a common body weight (930±20)

| Items | Cholesterol (mmol $L^{-1}$) | CK (U $L^{-1}$) | Glucose (mmol $L^{-1}$) | Total protein (g $L^{-1}$) | Na (mmol $L^{-1}$) | K (mmol $L^{-1}$) | Cl (mmol $L^{-1}$) | LDH (U $L^{-1}$) | HLR |
|---|---|---|---|---|---|---|---|---|---|
| **Genotype** | | | | | | | | | |
| †RJF | 3.52 | 4245.00 | 12.48 | 53.79$^{a}$ | 150.20$^{a}$ | 6.07 | 106.79 | 1270.00 | 0.384$^{b}$ |
| VF | 3.12 | 3808.00 | 12.37 | 40.02$^{b}$ | 146.92$^{b}$ | 5.81 | 106.42 | 1205.00 | 0.892$^{a}$ |
| CB | 3.19 | 3717.00 | 11.96 | 28.97$^{c}$ | 150.36$^{a}$ | 5.90 | 108.62 | 1303.00 | 0.850$^{a}$ |
| Stage of heat treatment | | | | | | | | | |
| No heat | 3.21 | 3935.00 | 11.53$^{b}$ | 41.73 | 150.32$^{a}$ | 6.44$^{a}$ | 107.47 | 1274.00 | 0.601$^{b}$ |
| 3 h heat at 36°C | 3.35 | 3888.00 | 13.05$^{a}$ | 39.15 | 147.82$^{b}$ | 5.37$^{b}$ | 107.10 | 1243.00 | 0.815$^{a}$ |
| SEM | 0.09 | 182.18 | 0.33 | 1.49 | 0.59 | 0.16 | 0.47 | 39.21 | 0.050 |
| **Probabilities** | | | | | | | | | |
| ANOVA | | | | | | | | | |
| Genotype | NS | NS | NS | ** | * | NS | NS | NS | ** |
| Stage of heat treatment | NS | NS | ** | NS | * | ** | NS | NS | ** |
| Genotype x stage of heat treatment | NS | * | ** | NS | NS | NS | NS | * | NS |

†RJF: Red Jungle Fowl, VF: Village Fowl, CB: Commercial Broiler; NS-Not Significant; *p<0.05; **p<0.01; $^{a,b,c}$Means with no common superscripts within a column-subgroup differ significantly

Table 4: Mean±SEM serum Creatine Kinase (CK), glucose and Lactate Dehydrogenase (LDH) where genotype x stage of heat treatment interactions were significant at a common body weight (930±20)

| Results | †RJF | VF | CB |
|---|---|---|---|
| **Creatine kinase (U $L^{-1}$)** | | | |
| No heat | 4446±422$^{a}$ | 4308±349$^{a}$ | 3050±499$^{b}$ |
| 3 h heat at 36°C | 3994±544 | 4009±251 | 3383±475 |
| **Glucose (mmol $L^{-1}$)** | | | |
| No heat | 12.56±0.40 | 12.78±0.28 | 11.81±0.78$^{y}$ |
| 3 h heat at 36°C | 12.40±0.64$^{b}$ | 13.32±0.39$^{ab}$ | 14.41±0.26$^{ax}$ |
| **LDH (U $L^{-1}$)** | | | |
| No heat | 1422±55$^{x}$ | 1188±115 | 1266±122 |
| 3 h heat at 36°C | 1099±83$^{by}$ | 1221±85$^{ab}$ | 1380±70$^{a}$ |

†RJF: Red Jungle Fowl, VF: Village Fowl, CB: Commercial Broiler; $^{a,b}$Means±SEM within a row with no common letters differ at p<0.05; $^{x,y}$Means±SEM within a column with no common letters differ at p<0.05

high growth rate (Mitchell and Sandercock, 1995; Sandercock *et al*., 2001). Although, LDH have been used as indicators of cell permeability resulting from muscle damage (Feng *et al*., 2008), the present findings suggested that the enzyme responded differently to heat treatment in RJF. There is no clear explanation to the phenomenon although, it has been reported that there is a positive correlation between thyroxine with serum LDH activity (Nazifi *et al*., 2003; Kataria *et al*., 2008). Hence, there is a possibility that RJF may be able to reduce thyroxine production more efficiently than VF and CB under high ambient temperature.

The elevated blood glucose level in CB following heat treatment in experiment 2 is consistent with earlier studies (Zulkifli *et al*., 2000; Kataria *et al*., 2008). Elevation in blood glucose level may be attributed to increase in glucocorticoids secretion which plays a major role in glucose metabolism (Freeman, 1971). On the contrary, Sahin *et al*. (2002) and Nazifi *et al*. (2003) reported that heat stress resulted in hypoglycemia. The researchers attributed the decline in blood glucose concentration during heat stress to a decrease in concentration of thyroxine, which is closely associated with energy metabolism during heat stress. Reduction in serum thyroxine is probably to lower metabolic rate for thermoregulation and to prevent hyperthermia. Serum total protein concentration of RJF, VF and CB were similar when compared at a common age (experiment 1) but not at a common body weight (experiment 2). In experiment 2, RJF had consistently higher total protein concentration than VF and CB. These results can be associated with age (Sribhen *et al*., 2003).

Literature regarding the influence of high ambient temperature on plasma electrolyte status in chickens is conflicting. While Khone and Jones (1975) reported increased blood K in response to heat stress, Ait-Boulahsen *et al*. (1995), Boeges *et al*. (2004) and Zulkifli *et al*. (2007) noted otherwise. Lin *et al*. (2000) reported wide variations in plasma parameter indices, Cl content was increased by high temperature and sodium and potassium content was decreased by high temperature. A rise in hemodilution is considered as the reason for general lowering in electrolyte concentration after heat stress (Smith and Teeter, 1987). Interestingly, regardless of stage of heat treatment, serum Cl level was higher in the CB than VF and RJF (Table 2). It is thought that under high temperature and the resulting respiratory alkalosis more Cl is needed in body fluids to exert an acidic action to normalize blood pH (Boeges *et al*., 2004). In the present study, comparison at both common age and body weight suggested that the effect of heat treatment on serum Na, Cl and K varied according to genotype.

The main objective of the present study was to compare the effect of heat treatment on blood biochemistry and HLR in RJF, VF and CB. Based on serum glucose, CL and HLR, CB appears to be more susceptible to heat stress than RJF and VF when compared at a common age. This is expected because CB had greater body weights and the negative relationship between body size and heat tolerance has been well documented (Bohren *et al*., 1981; Zulkifli *et al*., 1999; Sandercock *et al*.,

2006). However, the present findings suggest that the difference in body weight between RJF and VF was insufficient to cause significant variations in response to heat stress. Differences in heat tolerance between genotypes can be confounded with differences in body weight. Zulkifli *et al.* (1999) suggested that a common body weight as a point of reference for genotypes known to differ in growth pattern may provide insights into the true differences.

The reserachers reported that at a common age (with large disparity in body weight), RJF had lower rises in HLR and body temperature than CB following heat challenge. However, the superiority of RJF over CB in the ability to withstand high temperature was not observed when compared at a common body weight. Findings in experiment 2 showed that although both genotypes had a similar body weight, CB had elevated serum levels of glucose and HLR compared to their RJF counterparts which suggest that the former is more susceptible to heat stress. Thus, it appears that irrespective of age and body size, RJF are more tolerant to high temperature than CB. It is interesting to note that even though Zulkifli *et al.* (1999) compared RJF and CB of a similar age (150 days) and approximately a similar body weight (1000 g versus 930 g) their results are in disagreement with ours.

The researchers concluded that RJF were more heat tolerant than CB because of their smaller body size. There appears to be no obvious explanation for the apparent discrepancies albeit it could be associated with differences in the gender of birds used and protocol of heat treatment practiced. In general, comparison at a common age and a common body weight revealed no obvious differences in the response of RJF and VF to heat treatment. The findings suggest that although VF has undergone domestication, their ability to withstand high ambient temperature has not been affected. Such ability is critical for the VF because they are commonly raised in free-range system. Poultry breed and strain differences in tolerance to high temperature have been well documented (Horst, 1989; Deeb and Cahaner, 1999).

There is some evidence that genetic variation in thyroid activity could be associated with genetic variation in response to heat stress (Bowen and Washburn, 1984). The researches indicated that white leghorns were able to reduce their basal metabolic rate to a greater degree than heavy breed under heat stress condition. Since thyroxine has a profound influence on basal metabolic rate it may suggest a greater reduction in thyroid activity. It is not clear whether the superiority of RJF and VF over CB in tolerating heat stress could be related to thyroid activity. There is a growing body of evidences to suggest the profound role of heat shock proteins in enhancing heat tolerance in poultry (Zulkifli *et al.*, 2003; Liew *et al.*, 2003). In a heat shocked cell, these proteins may bind to heat sensitive proteins and protect them from degradation or may prevent damaged proteins from immediately precipitating and permanently affecting cell viability (Etches *et al.*, 1995). The possible variation in heat shock protein response of RJF, VF and CB to high ambient temperature merits further investigation.

## CONCLUSION

In this study, the selection for rapid growth for many decades has resulted in tremendous alterations in the anatomy and physiology of commercial broiler chickens and concomitantly the ability to withstand high ambient temperature as compared to the red jungle fowl and indigenous village fowl. It is also apparent that genetic differences in body size per sec may not determine breed or strain variations in response to heat stress.

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