GENETICS

Physiological responses of 3 chicken breeds to acute heat stress

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ABSTRACT Domestic animals have been modified by selecting individuals exhibiting desirable traits and culling the others. To investigate the alterations introduced by domestication and selective breeding in heat stress response, 2 experiments were conducted using Red Jungle Fowl (RJF), village fowl (VF), and commercial broilers (CB). In experiment 1, RJF, VF, and CB of a common chronological age (30 d old) were exposed to 36 ± 1°C for 3 h. In experiment 2, RJF, VF, and CB of common BW (930 ± 15 g) were subjected to similar procedures as in experiment 1. Heat treatment significantly increased body temperature, heterophil:lymphocyte ratio, and plasma corticosterone concentration in CB but not in VF and RJF. In both experiments and irrespective of stage of heat treatment, RJF showed lower heterophil:lymphocyte ratio, higher plasma corticosterone concentration, and higher heat shock protein 70 expression than VF and CB. It can be concluded that selective breeding for phenotypic traits in the domestication process has resulted in alterations in the physiology of CB and concomitantly the ability to withstand high ambient temperature compared with RJF and VF. In other words, domestication and selective breeding are leading to individuals that are more susceptible to stress rather than resistant. It is also apparent that genetic differences in body size and age per se may not determine breed or strain variations in response to heat stress.

Key words: heat stress, Red Jungle Fowl, village fowl, broiler chicken, heat shock protein

2011 Poultry Science 90:1435–1440
doi:10.3382/ps.2011-01381

INTRODUCTION

The most notable effect of domestication and selective breeding on physiology and behavior is reduced responsiveness to stress-evoking stimuli (i.e., environmental stress) as an adaptation to living in a biologically safe, predator-free environment (Korte et al., 2005). In pigs, considerable differences between wild and domesticated genotypes have been reported in plasma cortisol levels both in basal conditions and following stress, with the highest levels being found in wild boars (Weiler et al., 1998). The researchers concluded that the high growth potential of domesticated genotypes is mainly attributable to lower levels of circulating cortisol. Besides the complex physiological roles of glucocorticoids as primary mediators of allostasis and animal welfare (reviewed by McEwen and Wingfield, 2003; Korte et al., 2007), heat shock protein (**Hsp**) 70 is known to be an important modulator and indicator of the stress response. Ulmasov et al. (1992) showed that lizards inhabiting deserts are characterized by a higher expression of Hsp 70 at normal physiological temperatures (2- to 5-fold differences) when compared with those from colder regions. The expression of Hsp 70 in response to stress serves to protect against the initial insult, augment recovery, and produce a state of resistance to subsequent stress (Gerner and Schneider, 1975; Kregel, 2002). Mazzi et al. (2003) analyzed the Hsp 70 gene in broilers exposed to heat stress and found polymorphic sites located upstream from the coding region. Though a wealth of literature exists that suggests breed and strain differences in tolerance to heat stress in chickens (Yahav et al., 1998; Deeb and Cahaner, 1999; Cahaner et al., 2008), the Hsp 70 expression in the Red Jungle Fowl (**RJF**), the ancestor of domestic fowl, remains to be investigated. It is generally accepted that slower growing strains of native or indigenous breeds of chickens in tropical countries are better able to withstand high ambient temperatures than faster growing strains (Horst, 1989; Yunis and Cahaner, 1999). Red Jungle Fowl that inhabit the warmest and humid parts of Asia are highly adaptable to hot and humid conditions. Zulkifli et al. (1999) compared the physiological responses to heat treatment in RJF and commercial broiler chickens (**CB**) at a common age and at a common BW. When comparison was made at a common age (with large disparity in BW), the RJF unlike CB

Received January 25, 2011.
Accepted March 19, 2011.
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had no significant increases in heterophil:lymphocyte ratios (**HLR**), an indicator of physiological stress reaction in avian species. However, comparison at a common BW revealed no superiority among RJF over CB in the ability to withstand high ambient temperature. Zulkifli et al. (1999) and Sandercock et al. (2006) concluded that considerable attention should be given to genetic differences in BW in studies of a genotype effect on heat stress in poultry. Given the importance of glucocorticoids and Hsp 70 in physiological reaction to high ambient temperatures, the present study investigated the effect of domestication on Hsp 70 expression and other physiological parameters among CB, VF, and RJF in response to heat exposure at a common BW and a common chronological age.

MATERIALS AND METHODS

Birds and Husbandry

The study was undertaken following the guidelines of the Research Policy on animal ethics of the Universiti Putra Malaysia. A total of 40 female CB (Cobb 500), 40 female indigenous village fowl (**VF**), and 40 female RJF (*Gallus gallus spadiceus*) were used in the study. The CB chicks were sexed by feather sexing, whereas the sex of the RJF and VF chicks was determined by cloacal examination at hatch. The 1-d-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, 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 indigenous VF was the descendant of the southeast Asia RJF that has been domesticated in villages in Malaysia through natural mating and selection over a long period of time (Ramlah, 1996).

Birds (according to genotype) were reared in groups of 5 in battery cages with wire floors. Allotted floor space was 1,107 cm^2/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 3,000 kcal of ME/kg) and finisher (crumble form; 20% CP and 3,200 kcal of ME/kg) diets from d 1 to 30 and d 31 onwards, respectively. Feed and water were available at all times, and the birds were provided 12 h of natural lighting.

Experiment 1

A total of 20 birds/genotype were used in experiment 1 (Figure 1). At 30 d of age (0830 h; ambient temperature: 26°C), 10 birds from each genotype (mean BW: RJF, 150 ± 7 g; VF, 354 ± 11 g; CB, 1,432 ± 24 g) were randomly chosen and gently removed with minimum disturbance to flock mates. Immediately following capture, blood samples were obtained via the brachial vein with EDTA as the anticoagulant for determination of HLR and plasma corticosterone concentration (**CORT**). Bird was caught and sampled one immediately after another. The catching and bleeding procedure did not exceed 1 min and should not influence CORT (Lagadic et al., 1990; Romero and Reed, 2005). Blood smears were prepared using Wright's stain and heterophils and lymphocytes were counted to a total of 60 cells (Gross and Siegel, 1983). Blood samples for hormone assay were centrifuged and plasma was stored at −20°C until assayed. The CORT was measured using a sensitive and highly specific RIA kit (MP Biomedical, Irvine, CA; Perkin-Elmer 1470 Wizard Automatic Gamma Counter, Waltham, MA). Immediately following blood sampling, rectal body temperature (**Tb**) was measured by a digital thermometer (±0.1°C; Huger Electronics GmbH, Baden-Württemberg, Germany) with an insertion probe and recorded when the reading was stable for 15 s. After recording of Tb, the birds were decapitated and the entire brain was removed, frozen quickly in liquid nitrogen, and stored at −70°C until further analysis for Hsp 70 density.

The remaining birds (10 birds/genotype) were crated (10 birds/crate; crate dimension: 0.80 m × 0.60 m × 0.31 m), transferred to an environmentally controlled chamber, and exposed to 36 ± 1°C for 3 h. Neither feed nor water were provided during the heat treatment. Immediately after heat treatment, blood and brain samples were collected and rectal Tb were recorded as described earlier.

Brain samples (0.5 g) were homogenized in a Potter Elvehjem tissue grinder (Sigma, St. Louis, MO) using 3 mL of chilled Tris buffer (20 m*M* Tris, pH 7.5; 0.75 *M* NaCl; 2 m*M* 2-mescaptoethanol) with 10 μL/mL of protease inhibitor cocktail (lot no. P8340, Sigma Chemical Co., St. Louis, MO) and centrifuged at 23,000 × *g* for 45 min at 4°C. The protein concentrations of the supernatants were quantified by the bicinchoninic acid protein assay kit procedure (BCA-1, B9643, Sigma-Aldrich, St. Louis, MO) with BSA as the standard. Total protein (25 μg) was loaded and separated on 10% polyacrylamide gels (0.75 mm × 70 mm × 80 mm) containing SDS using a mini gel apparatus (Cleaver Scientific Ltd., Warwickshire, UK). Gels were electrophoresed at 120 V until the tracking dye reached the base of the gel. The fractionated proteins were visualized by Coomasie Blue staining or transferred to polyvinylidene difluoride membranes (MSI, Westborough, MA) using Trans-Blot semidry electrophoretic transfer cell (Cleaver Scientific Ltd.). After washing the membrane with distilled water, the nonspecific binding sites were blocked by 10 mL of cold blocking buffer (Kirkegaard and Perry Labs Inc., Gaithersburg, MD) for 60 min. The membranes were incubated for 1 h with 5 mL of blocking buffer containing antiserum (monoclonal

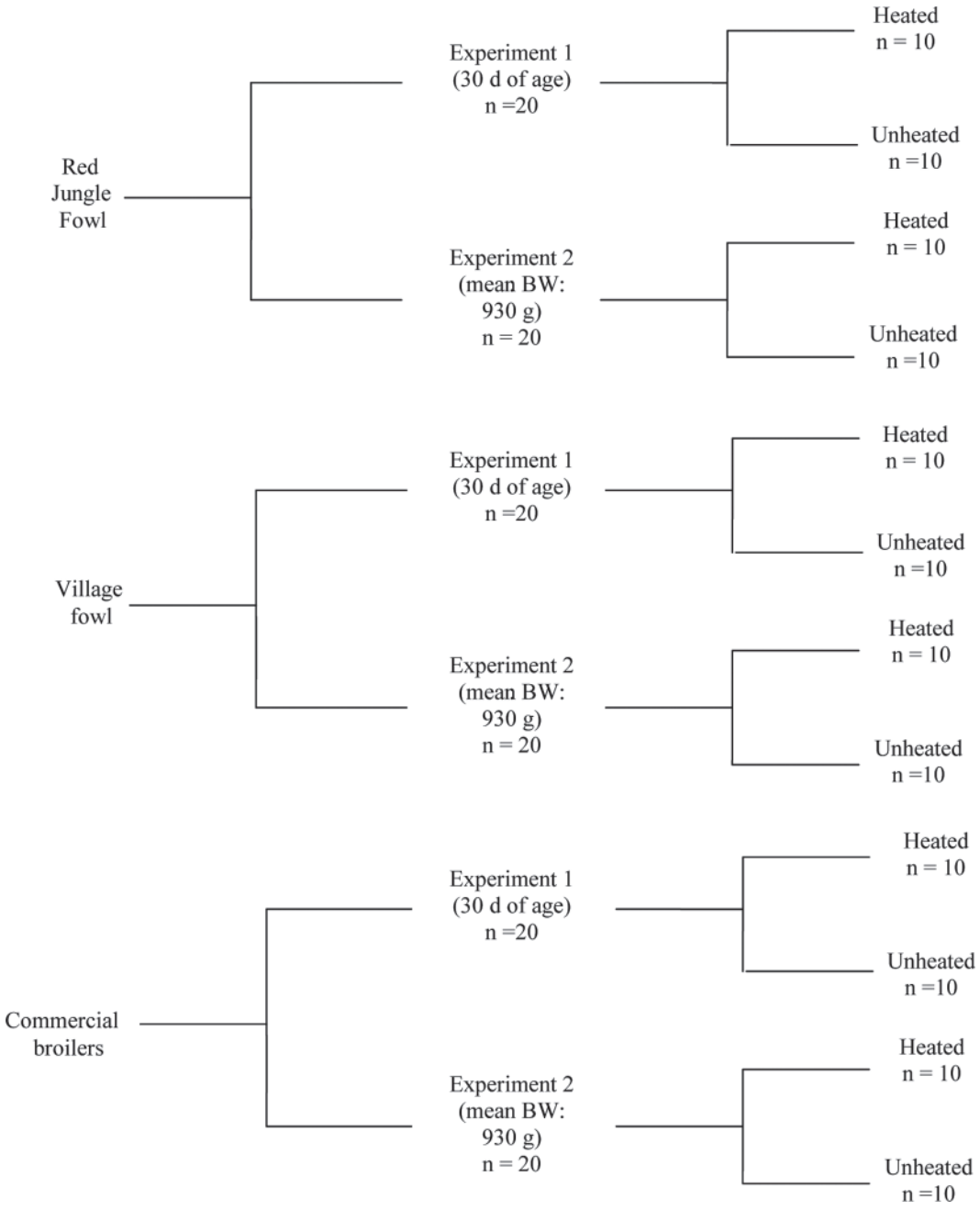

Figure 1. The experimental design.

mouse antibody; cat. no. ab6535, Abcam, Cambridge, MA) against Hsp 70 in a 1:20,000 dilution. Following 1 h of incubation, the blots were washed 3 times (5 min each) with 10 mL of cold Tris-buffered saline Tween 20. Blots were incubated in a horseradish peroxidase-conjugated rabbit antimouse secondary antibody for 30 min in a 1:40,000 dilution (cat. no. ab6728, Abcam). After rinsing with cold Tris-buffered saline Tween 20 (3 times, 5 min each) the blots were exposed to an enhanced chemiluminescent substrate (ChemiGlow, Alpha Innotech, San Leandro, CA). Visualization of bands was performed using a chemiluminescent imaging system (FluorChem 5500, Alpha Innotech) followed by quantification of the band summation intensity by Image-Pro Plus image processing and analysis software (Media Cybernetics, Silver Spring, MD). Sizes of the immunodetected proteins were confirmed by molecular weight markers (Precision Plus Protein, Bio-Rad, Hercules, CA). All solutions were made with Milli-Q water (Millipore, Bedford, MA).

Experiment 2

A total of 20 birds from each genotype (CB, 22 d of age; VF, 90 d of age; RJF, 150 d of age) of a common BW (930 ± 15 g) were used. Birds were subjected to heat treatment, blood and brain sampling, and recording of Tb as described in experiment 1 (Figure 1).

Statistical Analysis

Data were analyzed by 2-way ANOVA using SAS (SAS Institute, Cary, NC), with genotype (RJF, VF, and CB), stage of heat treatment (unheated and heated), and the interaction between them as main effects. Prior to analysis, CORT and HLR data were transformed to square roots. Untransformed means are presented in the tables. Comparisons among multiple means were made by Duncan's multiple range test. When interactions between main effects were significant, comparisons were made within each experimental variable. Statistical significance was considered as $P < 0.05$.

RESULTS

Experiment 1

Significant genotype × stage of heat treatment interactions were noted for Tb, HLR, CORT, and Hsp 70 expression (Table 1). Prior to heat treatment, genotype had no significant effect on Tb. However, following heat treatment, CB were more hyperthermic than their RJF and VF counterparts. Heat treatment resulted in a dramatic increase in HLR of CB but not in RJF and VF. The HLR of RJF was consistently lower than that of the other genotypes throughout the study. Irrespective of stage of heat treatment, the CORT of RJF was significantly higher than that of CB and VF. The CORT of RJF and VF were not significantly affected by the heat treatment. Prior to heat treatment, the Hsp 70 expression of RJF was significantly greater than that of the other genotypes. However, the heat treatment significantly augmented Hsp 70 expression in CB when compared with RJF and VF.

Experiment 2

Genotype × stage of heat treatment interactions were significant for Tb, HLR, CORT, and Hsp 70 expression (Table 2). In general, as noted in experiment 1, heat treatment significantly resulted in higher Tb, HLR, and CORT in CB but not in RJF and VF. Genotype had no significant effect on Tb before heat treatment. However, at high temperature, CB had the highest Tb. Regardless of stage of heat treatment, RJF had lower HLR and higher CORT than the other 2 genotypes. Under unheated conditions, RJF had significantly greater Hsp 70 expression than their VF and CB counterparts. Following heat treatment, the Hsp 70 expression of RJF was not significantly different from CB but greater than VF.

DISCUSSION

When discussing the results of the experiment, it should be remembered that during heat challenge the birds were crated, which itself may be a stressor. Thus, the birds were subjected to the stress of crating and heating simultaneously. However, work by Zulkifli et al. (2009) suggested that exposure to 34°C resulted in higher HLR than crating in broiler chickens. Based on Tb, HLR, CORT, and Hsp 70, CB were more susceptible to heat stress than RJF and VF when compared at a common age. This was expected because CB were heavier and the negative relationship between body size and heat tolerance has been well documented (Wilson et al., 1975; Sandercock et al., 2006). However, the present findings suggested that the difference in BW between RJF and VF was insufficient to cause a significant difference in Tb. Differences in heat tolerance between genotypes can be confounded with differences in BW. Zulkifli et al. (1999) suggested that a common BW as a point of reference for genotypes known to differ in growth pattern may provide insight into the true differences This was because at a common age (with large disparity in BW), RJF had lower increases in HLR and Tb than CB following the heat treatment. However, the superiority of RJF over CB in the ability to withstand high temperature was not observed when compared at a common BW.

Table 1. Mean (±SEM) deep body temperature (Tb), brain heat shock protein 70 (Hsp70) expression, plasma corticosterone concentration (CORT), and blood heterophil:lymphocyte ratio (HLR) where genotype × stage of heat treatment interactions were significant at a common age (30 d)[1]

Item	RJF	VF	CB
Tb (°C)			
Unheated	41.70 ± 0.10	41.95 ± 0.08	41.59 ± 0.16^{y}
Heated	41.62 ± 0.07^{b}	41.91 ± 0.09^{b}	43.65 ± 0.15^{a,x}
Hsp70 (summation density)			
Unheated	6,075 ± 159^{a}	5,241 ± 199^{b,y}	5,390 ± 185^{b,y}
Heated	6,269 ± 171^{b}	5,919 ± 122^{b,x}	7,014 ± 167^{a,x}
CORT (ng/mL)			
Unheated	12.17 ± 1.43^{a}	4.84 ± 0.45^{b}	2.28 ± 0.36^{c,y}
Heated	12.08 ± 2.42^{a}	6.22 ± 0.67^{b}	6.01 ± 0.94^{b,x}
HLR			
Unheated	0.31 ± 0.05^{b}	0.77 ± 0.14^{a}	0.70 ± 0.06^{a,y}
Heated	0.33 ± 0.02^{c}	0.83 ± 0.05^{b}	1.34 ± 0.18^{a,x}

$^{a–c}$Means ± SEM within a row with no common superscript differ at $P < 0.05$.

^{x,y}Means ± SEM within a column with no common superscript differ at $P < 0.05$.

[1]RJF: Red Jungle fowl; VF: Village fowl; CB: commercial broiler.

Table 2. Mean (±SEM) deep body temperatures (Tb), brain heat shock protein 70 (Hsp70) expression, plasma corticosterone concentration (CORT), and blood heterophil:lymphocyte ratio (HLR) where genotype × stage of heat treatment interactions were significant at a common BW (930 ± 15 g)[1]

Item	RJF	VF	CB
Tb (°C)			
Unheated	41.73 ± 0.21	41.67 ± 0.11	41.74 ± 0.05^{y}
Heated	41.67 ± 0.10^{b}	41.82 ± 0.08^{b}	42.47 ± 0.06^{a,x}
Hsp70 (summation density)			
Unheated	5,587 ± 134^{a}	4,537 ± 173^{b}	4,846 ± 271^{b,y}
Heated	5,501 ± 105^{a}	4,540 ± 117^{b}	5,649 ± 113^{a,x}
CORT (ng/mL)			
Unheated	10.06 ± 1.51^{a}	5.81 ± 0.73^{b}	2.62 ± 0.25^{c,y}
Heated	10.64 ± 1.16^{a}	6.47 ± 0.71^{b}	5.32 ± 0.50^{b,x}
HLR			
Unheated	0.35 ± 0.03^{b}	0.73 ± 0.07^{a}	0.72 ± 0.05^{a,y}
Heated	0.42 ± 0.04^{c}	0.83 ± 0.09^{b}	1.19 ± 0.11^{a,x}

$^{a–c}$Means ± SEM within a row with no common superscript differ at $P < 0.05$.

^{x,y}Means ± SEM within a column with no common superscript differ at $P < 0.05$.

[1]RJF: Red Jungle fowl; VF: Village fowl; CB: commercial broiler.

Findings in experiment 2 demonstrated that although both genotypes had a similar BW, CB had increased Tb, HLR, CORT, and Hsp 70 compared with their RJF counterparts, which suggested that the former was more susceptible to heat stress. Thus, irrespective of age and body size, RJF appeared to be more tolerant to high temperature than CB. The findings of Zulkifli et al. (1999) who compared RJF and CB at a similar age (150 d) and approximately similar BW (1,000 g vs. 930 g) are in disagreement with the present results. The authors 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, although they could be associated with differences in the protocol of heat treatment practiced (6 h of heat exposure). The present finding also suggested that VF were more heat tolerant than CB. This could be because VF were domesticated in a warmer climate whereas CB underwent generations of selection in a temperate climate.

In the present study, RJF consistently showed higher CORT than VF and CB under both unheated and heated conditions. This phenomenon could be associated with the natural environment of the RJF, where the birds are exposed to predators and must continuously search for food and shelter (Rovee et al., 1977). Similarly, Weiler et al. (1998) showed that wild pigs had higher plasma cortisol levels than domesticated breeds under both normal and stressful conditions. According to the authors, cortisol is regarded as a key hormone of catabolic processes and the differences in cortisol secretion between wild and domestic pigs favor the hypothesis that selection for growth potential led to a reduced secretion of glucocorticoids.

Although the body of evidence on cellular and molecular protective roles of Hsp 70 in poultry is growing (Liew et al., 2003; Al-Aqil and Zulkifli, 2009), genotypic differences have not been elucidated. Consistent with Tb, HLR, and CORT data, the RJF did not show Hsp 70 reaction to heat treatment. However, heat treatment increased Hsp 70 synthesis in CB in both experiments 1 and 2 and VF in experiment 1. The lack of Hsp 70 response in VF in experiment 2 could be associated with the older age of the birds, which may increase the possibility of heat adaptation. The higher Hsp 70 expression in CB after heat treatment could be associated with their higher Tb (Zulkifli et al., 2003); thus, Hsp 70 response is considered a cellular thermometer (Craig and Gross, 1991). This phenomenon together with the increased CORT and HLR in CB after heat treatment demonstrated their greater susceptibility to heat stress compared with VF and RJF.

The RJF at a common age and common BW showed significantly higher levels of basal Hsp 70 compared with VF and CB. This striking finding can be explained by the higher level of basal CORT in RJF regardless of age and BW. Correlation analysis by Mahmoud et al. (2004) revealed a significant positive association between CORT and Hsp 70 expression in broilers subjected to cyclic heat stress. Consistent with our findings, Ulmasov et al. (1992) showed that lizards inhabiting the middle Asia deserts are characterized by a higher basal Hsp 70 expression at normal physiological temperatures (2- to 5-fold differences) when compared with those from central and northern regions of Russia. This phenomenon apparently reflects the readiness of such animals to react to abrupt changes in environmental temperature and the possible heat stress when they are active in hunting or escaping from a predator.

It appears that selective breeding has resulted in alterations in the physiology of CB and concomitantly the ability to withstand high ambient temperature compared with RJF and VF. Domestication and selective breeding are leading to individuals that are more susceptible to stress rather than resistant. It is also apparent that genetic differences in body size and age per se may not determine breed or strain variations in response to heat stress.

ACKNOWLEDGMENTS

We thank Per Jensen (IFM Biology, Division of Zoology, Linköping University, Sweden) for critical reading of the manuscript. We are also grateful to Shaik Mohamed Amin Babjee (Department of Veterinary Pathology and Microbiology, Faculty of Veterinary Medicine, Universiti Putra Malaysia) and Siti Nor Azha (Institute for Poultry and Livestock Development, Johor Baharu, Malaysia) for providing the RJF and VF chicks, respectively. This work was supported by the Ministry of Higher Education, Malaysia under the Fundamental Research Grant Scheme.

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