Acute heat-stress effects on physiology and fear-related behaviour in red jungle fowl and domestic fowl

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Zulkifli, I., Dass, R. T. and Che Norma, M. T. 1999. **Acute heat-stress effects on physiology and fear-related behaviour in red jungle fowl and domestic fowl**. Can. J. Anim. Sci. **79**: 165–170. Two experiments were conducted to compare heat tolerance of red jungle fowl (RJF) and commercial broilers (CB) at (i) a common age and (ii) a common body weight. In exp. 1, RJF and CB of a common age (8 wk) were exposed to 36 ± 1°C for 6 h. RJF had smaller increases in heterophil to lymphocyte ratios and body temperature and higher serum cholesterol concentrations than CB in response to the heat treatment, while tonic immobility (TI) measurements were not affected by heat stress. In exp. 2, RJF and CB of a common body weight (1000 ± 150 g) were subjected to similar procedures as in exp. 1 (except TI durations). Both breeds responded similarly to the heat treatment. In both experiments, RJF had higher serum glucose level than CB, and the trait was not affected by heat exposure.

Key words: Heat stress, physiology, fear, jungle fowl, broilers

Zulkifli, I., Dass, R. T. et Che Norma, M. T. 1999. **Effet d'un stress thermique aigu sur la physiologie et sur le comportement de crainte chez les galliformes rouges d'Asie et chez la poule domestique**. Can. J. Anim. Sci. **79**: 165–170. Deux expériences ont été réalisées pour comparer la tolérance à la chaleur des galliformes rouges d'Asie (GRA) et des poulets de chair de production commerciale (PC) à un même âge et à un même poids corporel. Dans l'expérience 1, les GRA et les PC du même âge (8 semaines) étaient exposés à une température de 36 ± 1°C pendant 6 h. Les GRA manifestaient des accroissements moins importants du rapport hétérophiles-lymphocytes et de la température corporelle ainsi qu'une plus forte concentration sérique de cholestérol que les PC en réponse au traitement thermique. Par ailleurs, les valeurs d'immobilité tonique (IT) n'étaient pas touchées par le stress thermique. Dans l'expérience 2, les GRA et les PC de poids corporel comparable (1 000 ± 150 g) étaient exposés aux mêmes conditions que dans l'expérience 1, excepté pour la durée de IT. Les deux types de volaille réagissaient de la même façon au traitement thermique. Dans les 2 traitements les GRA avaient un niveau sérique de glucose plus élevé que les poulets domestiques et ce caractère se maintenait en présence de stress thermique.

Mots clés: Stress thermique, physiologie, crainte, galliformes sauvages rouges d'Asie, poulets de chair

A review of the literature indicates that there are genetic components in response to high ambient temperatures in poultry (Smith and Oliver 1971; Washburn 1985; Gowe and Fairfull 1995). Reports indicate that fast-growing broilers are more susceptible to heat prostration than slow-growing strains (Washburn et al. 1980; Washburn and Pinson 1990; Eberhart and Washburn 1993b). Intense selection for rapid growth rate 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 possess some tolerance to heat stress and adaptability to tropical conditions (Horst 1988, 1989). There is, however, a paucity of information on heat resistance in jungle fowl, the ancestor of the domestic fowl (Crawford 1990). Siegel et al. (1992) and Dunnington et al. (1994) compared domestic and jungle fowl and found a considerable degree of genetic divergence between them, as measured by band sharing.

Most of the previous studies on breed and strain differences in response to high ambient temperature have emphasised comparisons at a set point in time. Chronological time as a standard for comparison has commonly been used to examine breed and strain differences in heat tolerance. However, because of the negative relationship between body size and heat resistance (Wilson et al. 1975; Washburn et al. 1980; Bohren et al. 1981; Eberhart and Washburn 1993a), genetic variations in body weight are possible confounding factors in studies of a breed effect on response to heat stress. Hence, a common body weight as a point of reference for breeds known to differ in growth pattern may provide new insights into true differences in heat tolerance among breeds.

Work carried out with both meat- and egg-type chickens suggests that the stress response attributable to beak trimming (Lee and Craig 1991), transportation (Mills and Nicol 1990), forced molting (Campo and Alvarez 1991) and social disruption (Jones and Faure 1982) may augment fearfulness. Although the neurochemistry of the fear response has not been clearly elucidated, it encompasses the adrenergic, dopamergic and cholinergic systems (Jones 1996), which also play a pivotal role in physiological stress responses

Abbreviations: **ANOVA**, analysis of variance; **CB**, commercial broilers; **CP**, crude protein; **EDTA**, ethylenediaminetetraacetate; **H**, heterophils; **L**, lymphocytes; **ME**, metabolizable energy; **RJF**, red jungle fowl; **SEM**, standard error of the mean; **TI**, tonic immobility

(Fillenz 1993; Stanford 1993). To the best of our knowledge, the only reported study on the effects of thermal stress on fearfulness in chickens is by Campo and Carnicer (1994). They indicated that heat-stressed laying hens were less fearful than controls, as measured by TI durations. Given the existence of breed and strain differences in fear response (Jones 1986a), additional studies are needed to further evaluate the relationship between heat stress and fear in other breeds of chickens.

The purpose of the present study was to compare heat tolerance of CB and RJF at (i) a common age and (ii) a common body weight. In addition, the effect of heat stress on TI durations was determined. We hypothesised that RJF, developed under hot and humid tropical conditions, are fully adapted to high temperatures and as such are able to tolerate acute heat stress better than CB, when compared at both points of references (common age; and a common body weight).

MATERIALS AND METHODS

General Methods

Male CB (AVIAN) and RJF were used in the study. The CB were obtained from a commercial poultry hatchery. The RJF breeding stock was originally captured from the secondary forest and oil palm plantations in peninsular Malaysia and were assumed to be genetically pure. Purity of the RJF was assessed by gross characteristics, namely, shape and size of the bird, colour of the plumage, colour of the shank and earlobes, pattern of arrangement of the tail feathers, and size and thickness of the comb (Vidyadaran 1987). The flock had adapted to domestication for the previous 2 yr and was maintained as a closed flock at Universiti Putra Malaysia. Birds were reared in groups of 10 in three-tiered battery cages with wire floors. Floor space allowed was 1107 cm^2 per $bird^{-1}$. The batteries were in a conventional open-sided house with cyclic temperatures (minimum, 25°C; maximum, 34°C). Relative humidity was between 75 and 90%. The chickens were vaccinated against Newcastle disease on days 7 and 21. Starter (crumble form, 21% CP and 2950 kcal ME kg^{-1}) and finisher (pellet form, 19% CP and 3100 kcal ME kg^{-1}) diets were provided from days 1 to 21 and day 22 onward, respectively. Water was available at all times, and birds were provided with 12 h natural lighting (intensity at birds' level was 63 lx) and 12 h supplementary lighting (intensity at birds' level was 10 lx).

Data were subjected to ANOVA, with main effects and their interactions in a factorial arrangement in a fixed-effect model. When interactions were significant, separate ANOVAs were conducted within each main effect. Prior to analysis, the data for TI durations were logarithmically (log_{10}) transformed. Results were considered statistically significant at $P \leq 0.05$ throughout. All analyses were made using SAS® computational package (SAS Institute Inc. 1991).

Experiment 1

At 8 wk of age, 15 CB (mean body weight, 2877 ± 40.6 g) and 15 RJF (mean body weight, 264 ± 10.2 g) were assigned at random within breed in groups of five to six battery cages with wire floors in a climatic chamber and heat stressed at 36 ± 1°C for 6 h. Floor space allowed was 2214 cm^2 $bird^{-1}$. Birds placed together in the chamber had been previously housed together. Relative humidity was not controlled, but measurements showed that it remained below 50%. Feed was not available throughout the duration of heat treatment. However, water was available ad libitum. Prior to heat treatment (0 h) and after 3 and 6 h of exposure, blood samples (via wing vein) (for H and L counts and serum glucose and cholesterol levels) and rectal temperature (via digital termometer inserted 3 cm into the rectum for 30 s) were taken from five birds of each breed. A different set of five birds was sampled on each occasion. Blood samples for H and L counts were collected with EDTA as the anticoagulant. 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). Blood samples for total glucose and cholesterol determination were serum separated and stored at –20°C. Analyses for total glucose and cholesterol were conducted on an automated spectrophotometer (Ultraspec®300, Cobas-Mira, Roche Diagnostic System, Basel, Switzerland) using standard diagnostic kits (Roche).

Immediately after each bleeding, the birds were carried to a separate room (no visual contact with other birds) and used for TI measurements. A modification of the procedure described by Benoff and Siegel (1976) was used. TI was induced as soon as the birds were caught by gently restraining them on their right side by the legs and wings for 15 s. The experimenter then retreated approximately 1 m and remained within 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 1986a). A stopwatch was started to record latencies until the bird righted itself. If the bird righted in less than 10 s, it was captured again and the restraining procedure was repeated. If TI was not induced after three trials the duration of TI was considered as 0 s. The maximum duration of TI allowed was 600 s (Campo and Carnicer 1993). All TI tests were conducted by the same experimenter.

Experiment 2

Fifteen CB (25 d of age) and 15 RJF (150 d of age) of a common body weight (1000 ± 15 g) were subjected to heat treatment as described in exp. 1. Except for durations of TI, procedures described in exp. 1 were repeated. TI response was not measured because the large difference in the ages of the two breeds is a possible confounding factor in the assessment of behaviour (Jones 1986a; Campo and Carnicer 1993).

RESULTS AND DISCUSSION

Experiment 1

There was a significant genotype × duration-of-heat-exposure interaction for body temperature (Fig. 1). While there were no differences between breeds prior to heat stress (0 h), CB had higher body temperatures than RJF following 3 and 6 h of exposure. After 3 h of heat treatment, for both breeds there was a marked increase in body temperature, which was maintained for the subsequent 3 h. Interaction of breed × duration of heat exposure was significant for serum

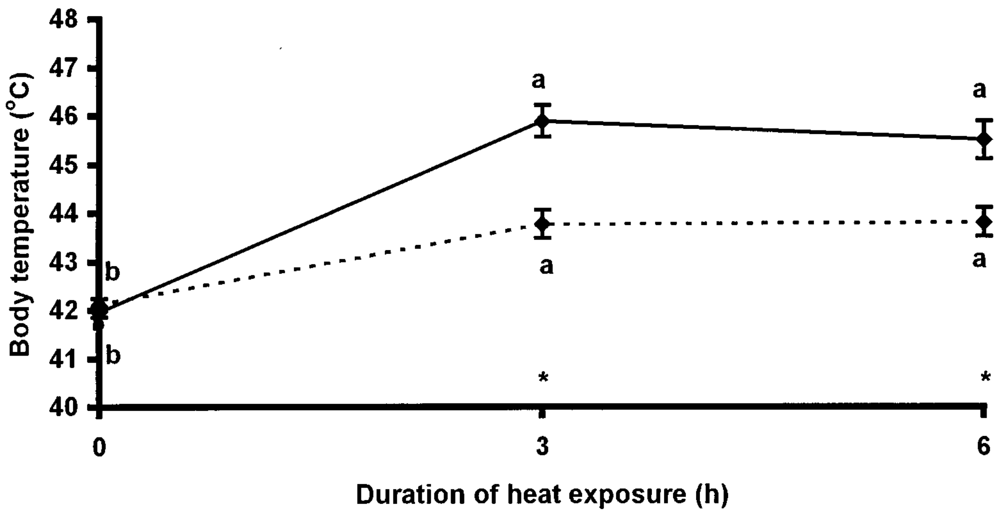

Fig. 1. Mean body temperatures (°C) at various durations of heat exposure where the breed (broilers [solid line] and jungle fowl [dashed line]) × duration-of-heat-exposure interaction was significant, exp. 1. a, b: Means within breed with no common letters differ at $P \leq 0.05$; * Differences between breeds are significant at $P \leq 0.05$.

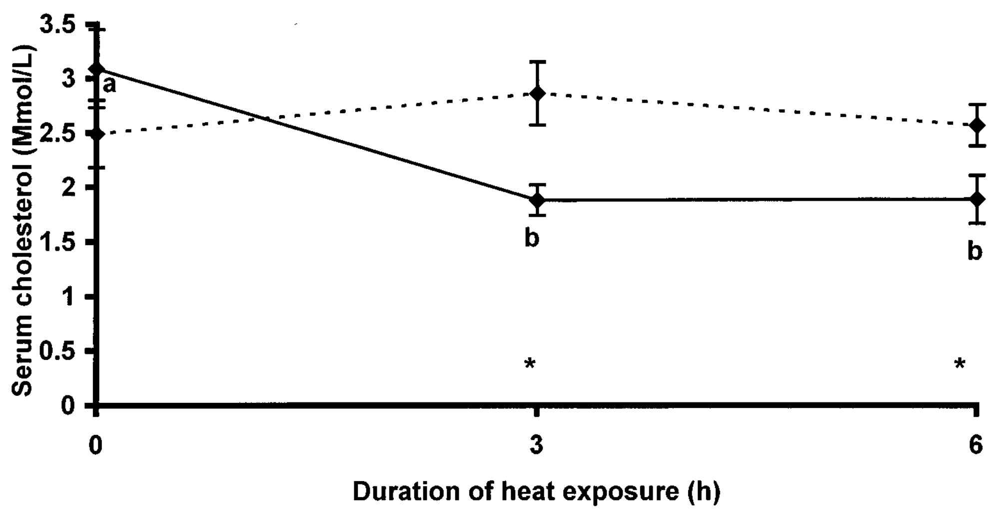

Fig. 2. Mean serum cholesterol levels (Mmol L^{-1}) at various durations of heat exposure where the breed (broilers [solid line] and jungle fowl [dashed line]) × duration-of-heat-exposure interaction was significant, exp. 1. a, b: Means within breed with no common letters differ at $P \leq 0.05$; * Differences between breeds are significant at $P \leq 0.05$.

cholesterol levels (Fig. 2). The interaction was caused by different effects of heat stress on the cholesterol concentration of CB and RJF. A significant decline in serum total cholesterol was detected in CB following heat exposure, whereas the heat treatment had no effect on the trait in RJF. Serum total glucose concentrations were greater for RJF (11.70 ± 0.63 Mmol L^{-1}) than CB (8.77 ± 0.60 Mmol L^{-1}). Mean serum glucose concentrations following 0 h (9.26 ± 0.94 Mmol L^{-1}), 3 h (10.95 ± 0.94 Mmol L^{-1}), and 6 h (10.51 ± 0.70 Mmol L^{-1}) of heat treatment were similar.

Comparison of CB and RJF revealed a significant breed × duration-of-heat-exposure interaction for H/L ratios (Fig. 3). Prior to the onset of heat treatment, breed had no significant effect on H/L ratios; however, CB exhibited a higher rise in H/L ratios at 3 and 6 h of heat exposure than RJF.

No statistical analyses can be applied successfully to assess the effect of breed on the recorded TI durations because the score of RJF always exceeded the test ceiling of 600 s. However, regardless of duration of heat exposure, there was a marked numerical difference, with RJF (600 ± 0.00 s) manifesting longer TI durations than CB (275 ± 43.63 s). TI durations were not affected by heat treatment (0 h, 439 ± 61.5 s; 3 h, 463 ± 62.3 s; 6 h, 410 ± 65.9 s).

The significant breed × duration-of-heat-exposure interactions for several traits measured during the heat treatment suggest that CB and RJF birds responded differently to a thermal stressor. Although the heat treatment resulted in hyperthermia in both CB and RJF, the latter were more efficient in regulation of body temperature. Elevated body temperature as a response to acute heat exposure has been

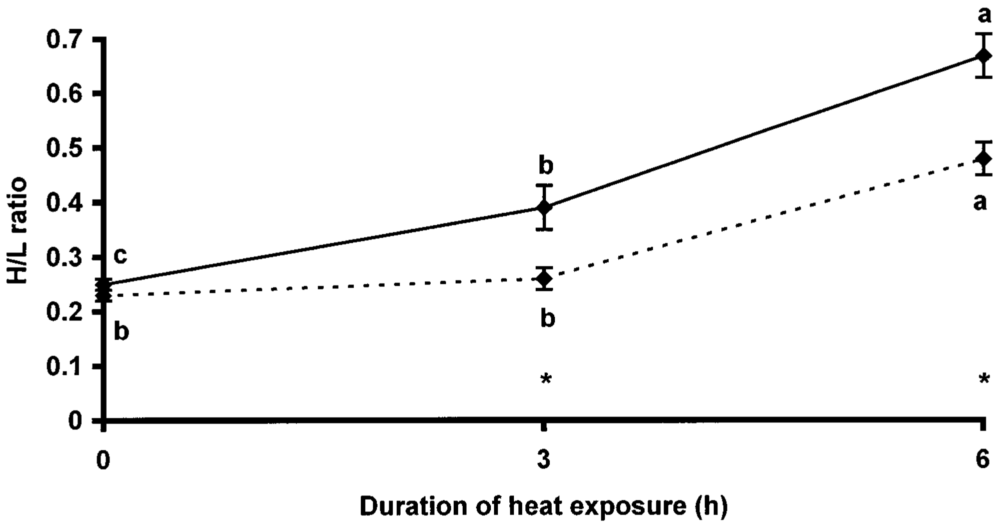

Fig. 3. Mean H/L ratios at various durations of heat exposure where the breed (broilers [solid line] and jungle fowl [dashed line]) × duration-of-heat-exposure interaction was significant, exp. 1. a, b Means within breed with no common letters differ at $P \leq 0.05$; * Differences between breeds are significant at $P \leq 0.05$.

demonstrated by Kohne et al. (1973) and Kutlu and Forbes (1993). El-Gendy and Washburn (1995) reported that birds with higher basal body temperature tended to have lower rises in body temperature during heat stress. In the present study, however, despite the lack of differences in normal body temperature between breeds, RJF had lower rates of body-temperature increase following heat exposure than CB.

H/L ratios appear to be a reliable measure of thermal stress in poultry (McFarlane and Curtis 1989; Zulkifli et al. 1994 a,b). One of the earliest studies to report on poultry blood leucocyte response to heat stress was that by Chancellor and Glick (1960). The authors noted an initial decrease in H and increase of L after exposure for 15–30 min. However, 2 h later the cellular numbers were reversed. In the present study, CB produced a rise in H/L ratios after 3 h of heat exposure, whereas a H/L-ratio response in RJF was noted after 6 h of heat treatment. The H/L ratios correlated well with body temperature, where CB exhibited a more dramatic response than RJF following heat exposure.

The results of this experiment indicate that acute heat stress may reduce serum cholesterol level. A similar response was noted in dairy cattle during the hot summer months (Shaffer et al. 1981). 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 (Alnaimy et al. 1992). The higher serum concentration of RJF during heat stress also suggests that RJF are better able to tolerate thermal stress than CB.

The profound impact of environmental stressors on fearfulness in poultry has been documented (Jones 1996). The lack of a heat-stress effect on TI durations is in disagreement with the findings of Campo and Carnicer (1994). Variation in genetic background, age and prior experiences (Jones 1986b) could be responsible for the discrepancies in the results obtained.

The differences in TI durations between breeds (Gallup et al. 1972; Jones and Faure 1981; Jones and Mills 1983) and strains within breed (Gallup 1974b; Benoff and Siegel 1976; Craig et al. 1983) suggest the importance of a genetic factor in fear-related behaviour in poultry. In the present study, it was apparent that RJF were more fearful than CB as assessed by a test of TI. However, it will be necessary to set a maximum TI duration of higher than 600 s to allow the TI data to be analysed statistically to find out with more certainty whether RJF are more fearful than CB. The augmented TI response of RJF could be attributed to the positive association between fearfulness and feather-pecking behaviour (Vestergaard et al. 1993). Zulkifli et al. (1998) studied harmful social behaviour in broilers and jungle fowl and noted high frequency of bird-to-bird pecking in the latter. Based on the results of the present experiment, it appeared that RJF were more heat tolerant than CB. However, it was not clear whether the better ability of RJF to withstand acute heat stress was attributable to superiority of genetic makeup or to the large disparity in body weight between the two breeds. To resolve this, the subsequent experiment compared RJF and CB at a common body weight.

Experiment 2

The effects of heat exposure on body temperature, serum cholesterol and glucose concentrations, and H/L ratios in exp. 2 are depicted in Table 1. There was no significant breed × duration-of-heat-exposure interaction for any of the parameters measured. Regardless of breed, 3 h of exposure to 36 ± 1°C resulted in a marked increase in body temperature, and it was not significantly changed thereafter. Heat treatment also affected H/L ratios and serum cholesterol concentrations. While there was no significant effect on either parameter following 3 h of heat exposure, by 6 h both CB and RJF exhibited a rise in H/L ratios and a decline in serum total cholesterol concentration. Serum glucose level

Table 1. Mean (± SEM) body temperatures, serum glucose and cholesterol concentrations, and H/L ratio by duration of heat exposure, exp. 2

	0 h	3 h	6 h
Body temperature (°C)	42.83 ± 0.13*b*	43.03 ± 0.12*a*	44.03 ± 0.26*a*
Serum glucose (Mmol L^{-1})	11.67 ± 0.99	12.19 ± 0.60	10.70 ± 0.89
Serum cholesterol (Mmol L^{-1})	2.69 ± 0.13*a*	2.51 ± 0.18*ab*	2.16 ± 0.16*b*
H/L ratio	0.25 ± 0.01*b*	0.26 ± 0.02*b*	0.48 ± 0.02*a*

a,b Means within a row with no common letters differ significantly ($P \leq 0.05$).

Table 2. Mean (± SEM) body temperatures, serum glucose and cholesterol concentrations, and H/L ratios, by breed, exp. 2

	CB	RJF
Body temperature (°C)	43.75 ± 0.22	43.47 ± 0.18
Serum glucose (Mmol L–1)	9.93 ± 0.61*b*	13.11 ± 0.49*a*
Serum cholesterol (Mmol L–1)	2.45 ± 0.13	2.46 ± 0.15
H/L ratio	0.34 ± 0.03	0.34 ± 0.03

a,b Means within a row with no common letter differ significantly ($P \leq 0.05$).

was consistent throughout the duration of heat exposure. Except for serum total glucose concentration, breed had no significant effect on any of the parameters measured (Table 2).

It has been reported that susceptibility to heat prostration increases with age (Smith and Oliver 1971). In the present study, however, despite the large disparity in chronological age, both CB and RJF of a common body weight responded similarly to the heat treatment, as measured by rectal temperature, H/L ratios and serum cholesterol concentration. Thus, it appears that the ability to withstand high ambient temperatures is more closely related to body size than to chronological age.

General Discussion

It is interesting to note that regardless of the duration of heat exposure, serum glucose concentration was higher in RJF than in CB. This phenomenon might be associated with lower adiposity in RJF compared to CB (Wall and Anthony 1995). It has been documented that plasma glucose was lower in fat than in lean chickens (Touchburn et al. 1981; Simon and Leclercq 1982). According to Simon and Leclercq (1982), selection for high abdominal weight increases insulin release and concomitant rate of glucose clearance.

At first sight it appears that RJF, the ancestors of the domestic fowl (Crawford 1990), which inhabits the warmest and humid parts of Asia, are highly adaptable to thermal insults. However, the literature regarding heat resistance in jungle fowl is conflicting. Hillerman and Wilson (1951) noted more efficient thermoregulation in jungle fowl than in leghorns, whereas Ramlah et al. (1976) reported otherwise. The most striking finding of the current work is its verification of the notion that innate differences in heat tolerance between breeds can be confounded with differences in body weight. In exp. 1, where comparison was made at a common chronological age (with large disparity in body weight), RJF had lower rises in H/L ratios and body temperature and higher serum cholesterol concentration than CB following heat exposure. Comparison at a common body weight, however, revealed no superiority among RJF over CB in the ability to withstand high ambient temperature. Thus, it appears that the differences in heat resistance between CB and RJF in exp. 1 is mainly attributable to the large disparity in body weight.

In the study of Washburn et al. (1980), in which broilers had their feed restricted so that their body weights were reduced and similar to those of a nonselected randombred population, broilers survived longer than the randombreds under heat-stress conditions. However, because fasting per se may enhance heat tolerance (McCormick et al. 1979; Teeter et al. 1987; Zulkifli and Fauzi 1996), inferences should be made with caution.

In conclusion, our data strengthen the notion that considerable attention should be given to genetic differences in body weight in studies of a breed effect on heat stress in poultry.

Alnaimy, A. Habeeb, M., Fayaz, I., Marai, M. and Kamal, T. H. 1992. Heat stress. Pages 27–48 *in* C. Phillips and D. Piggins, eds. Farm animals and the environment. CAB International, Wallingford, UK.

Benoff, F. H. and Siegel, P. B. 1976. Genetic analyses of tonic immobility in young Japanese quail (*Coturnix coturnix japonica*). Anim. Learn. Behav. **4**: 160–162.

Bohren, B. B., Carson, J. R. and Rogler, J. C. 1981. Response to selection in two temperatures for fast and slow growth to nine weeks of age. Genetics **97**: 443–448.

Campo, J. L. and Alvarez, C. 1991. Tonic immobility of several Spanish breeds of hens. Arch. Gefluegelkd. **55**: 19–22.

Campo, J. L. and Carnicer, C. 1993. Realized heritability of tonic immobility in White-Leghorn hens: a replicated single generation test. Poult. Sci. **72**: 2193–2199.

Campo, J. L. and Carnicer, C. 1994. Effects of several “stressors” on tonic immobility reaction of chickens. Arch. Gefluegelkd. **58**: 75–78.

Chancellor, L. and Glick, B. 1960. Effects of temperature as a stressor on white blood cells, adrenals and bursa of Fabricus of chicks. Am. J. Physiol. **198**: 1346–1348.

Craig, J. V., Craig, T. P. and Dayton, A. D. 1983. Fearful behaviour by hens of two genetic stocks. Appl. Anim. Ethol. **10**: 263–273.

Crawford, R. D. 1990. Origin and history of poultry species. Pages 1–42 *in* R. D. Crawford, ed. Poultry breeding and genetics. Elsevier, Amsterdam, The Netherlands.

Dunnington, E. A., Stallard, L. C., Hillel, J. and Siegel, P. B. 1994. Genetic diversity among commercial chicken populations estimated from DNA fingerprints. Poult. Sci. **73**: 1218–1225.

Eberhart, D. E. and Washburn, K. W. 1993a. Variation in body temperature response of naked neck and normally feathered chickens to heat stress. Poult. Sci. **72**: 1385–1390.

Eberhart, D. E. and Washburn, K. W. 1993b. Assessing the effect of the naked neck gene on chronic heat stress resistance in two genetic populations. Poult. Sci. **72**: 1391–1399.

El-Gendy, E. A. and Washburn, K. W. 1995. Genetic variation in body temperature and its response to short term acute heat stress in broilers. Poult. Sci. **74**: 225–230.

Fillenz, M. 1993. Neurochemistry of stress: introduction to techniques. Pages 248–279 *in* S. C. Stanford and P. Salmon, eds. Stress (from synapse to syndrome). Academic Press, London, UK.

Gallup, G. G., Jr. 1974. Genetic influence on tonic immobility in chickens. Learn. Behav. **2**: 145–147.

Gallup, G. G., Jr., Cumming, W. H. and Nash, R. F. 1972. The experimenter as an independent variable in studies of animal hypnosis in chicken (*Gallus gallus*). Anim. Behav. **20**: 166–169.

Gowe, R. S. and Fairfull, R. W. 1995. Breeding for resistance to heat stress. Pages 11–29 *in* N. J. Daghir, ed. Poultry production in hot climates. CAB International, UK.

Gross, W. B. and Siegel, H. S. 1983. Evaluation of heterophil/lymphocyte ratio as a measurement of stress in chickens. Avian Dis. **27**: 972–979.

Hillerman, J. P.and Wilson, W. O. 1951. Acclimation of adult chicken to environmental changes. Am. J. Physiol. **180**: 591–595.

Horst, P. 1988. Native fowl for a reservoir of genomes and major genes with direct and indirect effects on productive adaptability. Proc. 18th World's Poult. Congr., Nagoya, Japan. pp. 99–105.

Horst, P. 1989. Native fowl as a reservoir for genomes and major genes with direct and indirect effects on adaptability and their potential for tropically oriented breeding plans. Arch. Gefluegelkd. **53**: 93–101.

Jones, R. B. 1986a. The tonic immobility reaction of the domestic fowl: a review. World's Poult. Sci. J. **42**: 82–96.

Jones, R. B. 1986b. Response of domestic chicks to novel food as a function of sex, strain and previous experience. Behav. Proc. **12**: 261–271.

Jones, R. B. 1996. Fear and adaptability in poultry: insights, implication and imperatives. World's Poult. Sci. J. **52**: 131–174.

Jones, R. B. and Faure, J. M. 1981. Sex and strain comparisons of tonic immobility ("righting time") in the domestic fowl and the effects of various methods of induction. Behav. Proc. **6**: 47–55.

Jones, R. B. and Faure, J. M. 1982. Tonic immobility in the domestic fowl as a function of social rank. Biol. Behav. **7**: 27–32.

Jones, R. B. and Mills, A. D. 1983. Estimation of fear in two lines of the domestic chicks: correlation between various methods. Behav. Proc. **8**: 243–253.

Kohne, H. J., Boone, M. and Jones, J. E. 1973. The effect of feed consumption on the survival time of adult turkey hens under conditions of acute thermal stress. Poult. Sci. **52**: 1780–1783.

Kutlu, H. R. and Forbes, J. M. 1993. Changes in growth and blood parameters in heat stressed broiler chicks in response to dietary ascorbic acid. Livest. Prod. Sci. **36**: 335–350.

Lee, H. Y. and Craig, J. V. 1991. Beak trimming effects on behaviour patterns, fearfulness, feathering, and mortality among three stocks of White Leghorn pullets in cage floor pens. Poult. Sci. **70**: 211–221.

McCormick, C. C., Garlich, J. D. and Edens, F. W. 1979. Fasting and diet affect the tolerance of young chickens exposed to acute heat stress. J. Nutr. **109**: 1087–1097.

McFarlane, J. M. and Curtis, S. E. 1989. Multiple concurrent stressors in chicks. 3. Effects on plasma corticosterone and the heterophil and lymphocyte ratio. Poult. Sci. **68**: 522–527.

Mills, D. S. and Nicol, C. J. 1990. Tonic immobility in spent hens after catching and transport. Vet. Rec. **126**: 210–212.

Ramlah, H., Richards, S. A. and Sykes, A. H. 1976. Field observation on temperature regulation in four strains of domestic fowls. Malay. Agric. Res. **5**: 171–172.

Sandercock, D. A., Mitchell, M. A. and MacLeod, M. G. 1995. Metabolic heat production in fast and slow growing broiler chickens during acute heat stress. Br. Poult. Sci. **36**: 868–869.

SAS Institute, Inc. 1991. SAS/STAT® user's guide. Release 6.03. SAS Institute Inc., Cary, NC.

Shaffer, L., Roussel, J. D. and Koonce, J. L. 1981. Effect of age, temperature, season and breed on blood characteristics of dairy cattle. J. Dairy Sci. **64**: 62–70.

Siegel, P. B., Haberfeld, A., Mukherjee, T. K. A., Stallard, L. C., Marks, H. L. and Dunnington, E. A. D. 1992. Jungle-fowl – domestic fowl relationships: a use of DNA fingerprinting. World's Poult. Sci. J. **48**: 147–155.

Simon, J. and Leclercq, B. 1982. Longitudinal study of adiposity in chickens selected for high or low abdominal fat content: further evidence of a glucose–insulin imbalance in the fat line. J. Nutr. **112**: 1961–1973.

Smith, A. J. and Oliver, J. 1971. Some physiological effects of high environmental temperature on laying hens. Poult. Sci. **50**: 912–924.

Stanford, S. C. 1993. Monoamines in response and adaptation to stress. Pages 282–231 *in* S. C. Stanford and P. Salmon, eds. Stress (from synapse to syndrome). Academic Press, London, UK.

Teeter, R. G., Smith, M. O., Sangiah, S. and Mather, F. B. 1987. Effects of feed intake and fasting duration upon body temperature and survival of thermostressed broilers. Nutr. Reps. Int. **35**: 531–537.

Touchburn, S., Simon, J. and Leclercq, B. 1981. Evidence of a glucose–insulin imbalance and effect of dietary protein and energy level in chickens selected for high abdominal fat content. J. Nutr. **111**: 325–335.

Vestergaard, K. S., Kruijt, J. P. and Hogan, J. A. 1993. Feather pecking and chronic fear in groups of red jungle fowl: their relations to dust bathing, rearing environment and social status. Anim. Behav. **45**: 1127–1140.

Vidyadaran, M. K. 1987. Quantitative observations on the pulmonary anatomy of the domestic fowl and other ground-dwelling birds. Universiti Pertanian Malaysia, Malaysia. Ph.D. thesis.

Wall, C. W. and Anthony, N. B. 1995. Inheritance of carcass variables when giant jungle fowl and broilers achieve a common physiological body weight. Poult. Sci. **74**: 231–236.

Washburn, K. W. 1985. Breeding of poultry in hot and cold environments. Pages 111–122 *in* M. K. Yousef, ed. Stress phyiology in livestock. Vol. 3 – Poultry. CRC Publications, Boca Raton, FL.

Washburn, K. W. and Pinson, E. R. 1990. Research note: Variation in the three-week body temperature of broilers and Athens-Canadian randombred chickens. Poult. Sci. **69**: 486–488.

Washburn, K. W., Peavey, R. and Renwick, G. M. 1980. Relationship of strain variation and feed restriction in blood pressure and response to heat stress. Poult. Sci. **59**: 2568–2588.

Wilson, H. R., Wilcox, C. J., Voitie, R. A., Baird, C. D. and Dorminey, R. W. 1975. Characteristics of White Leghorn chickens selected for heat tolerance. Poult. Sci. **54**: 126–130.

Zulkifli, I. and Fauzi, A. M. 1996. Behaviour and performance of fasted broilers under high temperature and humidity. J. Vet. Malay. **8**: 1–6.

Zulkifli, I., Dunnington, E. A., Gross, W. B. and Siegel, P. B. 1994a. Food restriction early or later in life and its effect on adaptability, disease resistance, and immunocompetence of heat-stressed dwarf and nondwarf chickens. Br. Poult. Sci. **35**: 203–213.

Zulkifli, I., Dunnington, E. A., Gross, W. B. and Siegel, P. B. 1994b. Inhibition of adrenal steroidogenesis, food restriction and acclimation to high ambient temperatures in chickens. Br. Poult. Sci. **35**: 417–426.

Zulkifli, I., Babjee, S. A., Vidyadaran, M. K. and Ramlah, A. H. 1998. Relationship among growth, behaviour and stress response in broilers and red jungle fowl when reared separately or intermingled. Arch. Gefluegelkd. **62**: 150–155.