The effects of tropical environmental conditions on the stress and immune responses of commercial broilers, Thai indigenous chickens, and crossbred chickens

C. Tirawattanawanich,*1 S. Chantakru,† W. Nimitsantiwong,* and S. Tongyai*

*Department of Physiology, and †Department of Anatomy, Faculty of Veterinary Medicine, Kasetsart University, Bangkok 10900, Thailand

Primary Audience: Flock Supervisors, Researchers, Veterinarians

SUMMARY

Heat stress is a life-threatening factor in commercial broilers raised in the tropics (e.g., Thailand) without temperature-controlled housing facilities. However, these temperature-controlled facilities are hardly affordable by small-scale farmers. To avoid such limitations, a Thai indigenous crossbred line (C line), selected as a candidate meat-type chicken to survive in the tropical environment, was examined for its capacity to tolerate the tropical climate and for its immune functions. Comparisons, in terms of the stress and innate and humoral immune responses using the heterophil-to-lymphocyte ratio, abdominal exudative cell phagocytic activities, and serum anti-SRBC titer, were made between C-line chicks and the corresponding cohorts from their low-meat-yielding Thai indigenous (T line) parents and from high-meat-yielding commercial broiler (B line) chickens. The responses were evaluated in the 3 different seasons of Thailand (monsoon, summer, and winter). Significantly higher stress levels \( (P < 0.001) \), based on the heterophil-to-lymphocyte ratios, were detected in the B line chicks compared with those in the T and C lines at all ages regardless of the season, suggesting that the B line chicks were vulnerable to tropical heat stress whereas the T and C lines were well adapted, with no significant differences detected between the latter two. The innate and humoral immunities of B-line chicks were significantly lower \( (P < 0.001) \) than those of T- and C-line chicks. The differences were immense in the summer, when the immunity of the C- and T-line chicks outperformed that of the B-line chicks, with mean opsonized-SRBC phagocytic activities of 7.90, 8.31, and 4.74 and mean IgG titers of 8.00, 8.40, and 5.10, respectively. This could be a consequence of the heat stress causing immunosuppression in B-line chicks, and could represent a noteworthy adaptation to the tropical conditions of the C- and T-line chicks. The apparent climate-tolerant capacity conserved in the C-line chickens, with approximately 50% T genetics, could serve as a guideline for further genetic improvement toward a high-meat-yielding chicken that retains a suitable heat tolerance.

Key words: broiler, hybrid, immunity, Thai indigenous chicken, tropical stress

doi:10.3382/japr.2010-00190

1Corresponding author: fvetcnt@ku.ac.th
DESCRIPTION OF PROBLEM

Heat stress has various negative effects on livability, production performance, immune functions, and disease susceptibility in poultry [1–6]. Potential countermeasures to the adverse effects of heat stress have been suggested, such as the genetic selection for heat-tolerant genes [7] and tolerance induction by neonatal- or embryonic-age thermal conditioning [8–12] or by feed restriction [13–15]. Most studies, however, have been conducted under fixed high environmental temperature conditions. In contrast, very little information is available on the effects of the dynamic combination of temperature and RH that exists in actual tropical climates. A comprehensive understanding of, and hence a solution that is applicable to, the problem of tropical heat stress is therefore limited.

To ameliorate heat stress, the poultry industry in the tropics has utilized temperature-controlled housing facilities, such as evaporative cooling systems. A significant drawback is the high investment cost, which is barely affordable by small-scale farmers. Indeed, it is cost effective only if an intensive farming system is used. Such management systems, with the emphasis on high stocking density, lead to a major public concern for poor bird welfare, as indicated by some common health problems, such as footpad dermatitis, stress, and carcass injury [16–21], as well as an increased risk of disease outbreaks.

On the basis of bird welfare as well as from the socioeconomic standpoint mentioned above, future poultry production might resume the traditional open housing system without temperature-controlled facilities. Genetic improvements toward high-meat-yielding poultry would adversely affect bird heat tolerance and immunity, as exemplified by modern commercial broilers [4, 22, 23]. Heat stress has been reported to affect both cell-mediated and humoral immunity in chickens, and has been explored mostly by means of assaying phagocytic activities and serum antibody titers [24–27]. The development of genetic lines of chickens that can endure the tropical environment with the least compromise in terms of meat (or egg) production and immune performance is therefore in urgent demand.

Thai indigenous chickens (T line), which have evolved in a tropical environment and are well adapted to it, could play a major role in the development of a new commercial breed for rearing in tropical environments. In this study, a Thai-indigenous crossbred hybrid line (C line) was examined in terms of its heat tolerance and immunity in comparison with the parental T line and a commercial broiler line (B line).

MATERIALS AND METHODS

Experimental Design, Birds, and Sample Collection

The effects of tropical climate, represented by the summer (beginning of March to May), monsoon (beginning of July to September), and winter (end of October to December) seasons, and the chicken breed, represented by the T, B, and C lines, on heat stress and the immune responses were studied using a 3 × 3 factorial design, with season and chicken breed as the main effects. The bird housing facilities were prepared with 1 room divided by a mesh wall into 3 pens with the 3 different chicken lines assigned (i.e., 1 pen each for the T, C, and B lines). The pen sizes were adjusted to gain comparable floor density by taking into account different growth rates among breeds. Stocking density was calculated based on BW per square meter of floor space, at approximately 20 kg of BW/m². The BW used in the calculation were the anticipated BW gains by 6 wk of age according to the breeder information, which were 0.5, 1, and 2 kg for the T, C, and B chick lines, respectively. Daylight was allowed into the housing facility through opened glass windows installed along 1 side of the room, with each pen being equally exposed to daylight. The room was illuminated with ceiling lights during the dark period, in accordance with the standard broiler practice in Thailand. Ambient temperature and RH were recorded at all times by using a Sato thermohygrograph [28], and the data are presented as the mean, maximum, and minimum temperatures (Figure 1) and RH (Figure 2) of 3-d time intervals, derived from the averages of daily records. Identical records obtained from 3 thermohygrometers (1 placed in each pen) confirmed the uniformity of the ambient RH and temperature across the room between pens.

A total of 288 one-day-old, mixed-sex chicks were used in each of the 3 seasons, constituting 96 birds for each of the B, C, and T lines. All
groups were similarly fed commercial broiler feed (described below) and water ad libitum. Two feed rations were used: a starter diet providing 21% (wt/wt) CP and 3,100 kcal of ME/kg for the first 3 wk of age, and then a grower diet having 19% (wt/wt) CP and 3,150 kcal of ME/kg from 3 wk of age onward. The commercial broiler rations were formulated without antibiotic or coccidiostat supplementation.

Brooding was operated for the first 14 d of age using a 60-W incandescent light bulb as a heat source, according to the general practice of commercial broiler farming in Thailand. The vertical distance from the floor to the light bulb was adjusted during the day and night to the comfort of the B-line chicks, as justified by their normal behaviors, including feeding and movement. The distance used for the B-line chicks was similarly applied for the T- and C-line chick groups.

Stress levels were determined based on the heterophil-to-lymphocyte (H:L) cell ratios [29]. Immune responses were studied in 2 components, the nonspecific immune response, which was examined by measuring the abdominal exudative cell (AEC) phagocytic activity, and the humoral immune response, which was examined by monitoring the mercaptoethanol-sensitive and mercaptoethanol-resistant antibody titers (MES and MER, respectively).

At 2, 4, and 6 wk of age, 20 birds each per group (i.e., per B, T, and C line) were randomly selected for collection of a 0.5-mL blood sample for examination of the H:L ratio. At 6 wk
of age, another 12 birds were randomly selected for examination of the AEC phagocytic activity. The remaining 24 birds in each group were intravenously injected with 1 mL of 7% (vol/vol) SRBC at 6 wk of age and again at 7 wk of age, and then the serum anti-SRBC antibody titers were determined at 7 and 14 d after the second SRBC injection, using 12 birds each time. The blood samples were collected from the brachial vein, taking 0.5 and 1.5 mL for evaluation of the H:L ratio and serum agglutination titer, respectively. After sampling, the birds were tagged to prevent their later use and thus avoid the consequence of the sampling procedure on stress development. Signs of heat or cold stress (i.e., panting or huddling) were monitored by observation 6 times per day, at approximately 0600, 1000, 1200, 1400, 1800, and 2100 h, throughout the experimental period. The experimental birds were subjected to euthanasia for collection of abdominal fluid samples at the end of the experiments by using 100 mg/kg of BW of pentobarbital sodium via intravenous injection. The experimental protocol was approved by the Institutional Animal Care and Use Committee of Kasetsart University.

**H:L Ratio**

Fresh blood smears were prepared in triplicate on precleaned glass slides, air-dried, and stained with Wright’s stain. The slides were then observed under a light microscope. Objective fields were randomly chosen for examination of the number of heterophils and lymphocytes until a total of at least 100 cells on each slide were counted; the H:L ratio was then calculated. Care was taken to distinguish the small lymphocytes from thrombocytes based on the morphological characteristics.

**AEC Phagocytic Activity**

Abdominal exudative cell phagocytic activity was examined in the winter and summer seasons by applying the methods of Sabet et al. [30] and Trembicki et al. [31]. Sephadex G-50 [32] at 3% (wt/vol) in PBS (pH 7.4) was administered intraabdominally at 1 mL/100 g of BW, up to a maximum of 10 mL per bird, by using a 1-in. 23-gauge needle. The position of injection into the abdominal cavity was according to the suggestion of Sabet et al. [30]. While gently inserting the needle, a drawback on the plunger was cautiously examined to ensure that no blood, gut contents, or air bubbles were present in the syringe so as to confirm the appropriateness of the needle location and delivery of the Sephadex G-50. At 72 h postinjection, the birds were euthanized according to the aforementioned protocol, and the abdominal cavity was opened by making a small midline abdominal incision followed by blunt dissection to minimize the bleeding. The abdominal cavity was flushed 3 times with 10 mL each of sterile PBS (pH 7.4), and the harvested fluid was pooled in a sterile tube that was placed on ice for 20 min to allow sedimentation of tissue debris. The fluid was aspirated and centrifuged (400 × g, 10 min) at 4°C to separate the AEC. The pelleted AEC were then washed 3 times in PBS (pH 7.4) and reconstituted to a concentration of 1 × 10⁵ cells/mL in complete medium composed of Iscove’s Modified Dulbecco’s Medium and 5% (vol/vol) fetal bovine serum [33]. Approximately 10⁶ to 10⁷ cells were collected from each bird.

Opsonized SRBC were prepared using freshly collected SRBC in Alsever’s solution. The SRBC were washed 3 times in sterile PBS (pH 7.4), reconstituted to a 5% (vol/vol) concentration in PBS (pH 7.4), and opsonized by a 30-min incubation with a subagglutinating concentration of heat-inactivated chicken anti-SRBC serum. The opsonized SRBC were washed in sterile PBS (pH 7.4) and reconstituted as a 5% (vol/vol) suspension in complete medium. A suspension of 5% (vol/vol) unopsonized SRBC was prepared in the same manner except without incubation with the anti-SRBC serum.

To examine the phagocytic activity, a 100-µL drop of the AEC suspension on a coverslip was prepared in 2 sets of triplicate coverslips. The coverslips were left at room temperature for 30 min to allow cell-surface adhesion. Unattached cells were removed by washing 3 times in PBS (pH 7.4). One set of coverslips with adherent cells was overlaid with the 5% (vol/vol) opsonized SRBC, and the other set was overlaid with the unopsonized SRBC; both sets were then incubated at 38°C in a moisture chamber. After 2 h of incubation, the coverslips were washed 3 times in PBS (pH 7.4), fixed in absolute methanol, stained with Wright’s stain, and mounted.
on glass slides by using organic Permount [34]. A total of 100 cells were examined under a simple light microscope for phagocytic activity, from which the percentage of phagocytic macrophages present and the average number of phagocytosed SRBC per macrophage were determined.

**Serum Agglutination Titer**

The serum anti-SRBC titers were determined by hemagglutination test by applying the methods of Delhanty and Solomon [35] and Qureshi and Havenstein [23]. Two-fold dilutions of the serum samples were prepared in 2 sets of 96-well plates, 1 with β-mercaptoethanol and another without. Then 0.5% (vol/vol) SRBC was added and the agglutination reaction was observed after a 2-h incubation at room temperature. The MER was determined from the reaction in the presence of β-mercaptoethanol (0.05 M final concentration), whereas the MES was calculated by subtracting the MER from the agglutination titers without β-mercaptoethanol. The assay was performed in triplicate, and the results were averaged and recorded.

**Statistical Analysis**

The statistical significance of any differences between season, chicken breed, and their interaction was assessed by 2-way ANOVA using the GLM procedure of SAS [36]. When an effect was significant, a comparison of means was performed by Duncan’s multiple range test. Differences were considered significant at \( P < 0.05 \). Values are presented as the mean ± 1 SEM. The experimental unit was the individual bird.

**RESULTS AND DISCUSSION**

The goal of this study was to assess the effects of tropical environmental conditions on the stress and immune responses of 3 different (genetic) lines of chickens, with a specific focus on the innate and humoral immune functions. The tropical climate can best be described as hot and humid, and the ambient temperature and RH have been well documented as stress factors in chickens [6, 37, 38]. These reports, however, have been conducted under a certain constant temperature or RH that might not represent dynamic tropical environmental conditions, and the fluctuation in temperature, RH, or both may be important. Stress and immunity are among the key factors that affect the welfare and disease susceptibility of chickens, and these responses are influenced by genetics [27, 39, 40]. We therefore anticipate that the findings of this research can be offered as suggestions for the potential improvement of meat production-type chicken genetics more appropriate for rearing in tropical environments as well as in conditions complying with bird welfare regulations and concerns.

In this study, 3 different chicken (genetic) lines, ranging from high- to low-yield meat producers (i.e., the B, T, and C lines, respectively) were compared in the 3 seasons of Thailand (monsoon, summer, and winter). The housing and laboratory facilities and setting, the source of bird feed and feeding, and sample collection and testing were the same in all 3 experiments to avoid possible variations among experiments. Thus, ambient temperature and RH were used as explanatory factors contributing to the seasonal effect. It should be kept in mind that other environmental factors may partly influence the seasonal effect, such as wind velocity, which was excluded from this study because of the physical limitations of the laboratory building and may need to be scrutinized further. The effects of light intensity and day length were excluded because of the application of the commercial broiler practice in which electric lighting is provided, thereby neutralizing the effect of natural light. It is also important to note that differences in day length between seasons in Thailand were not more than 1 h, which are relatively small compared with those in the temperate zones.

**Stress Response**

The stress response was strongly influenced by both chicken breed and season \( (P < 0.001) \), with a highly significant interaction between both effects \( (P < 0.001) \), as shown in Table 1. It appeared that, overall, the stress levels in chicks of the B line were significantly higher than those in chicks of the C and T lines regardless of the season, and also that each of the 3 chicken (genetic) lines responded differently to the seasonal effects.
The H:L ratios, expressed in 2-wk-old B-line chicks, were found to increase significantly and equally in the monsoon and summer seasons compared with the winter, and were significantly higher than those present in the C- and T-line chicks in the monsoon and summer seasons. However, no significant differences among the 3 breeds were detected in the winter season. The H:L ratio has long been accepted as a stress index in avian species [41, 42]. Thus, it is plausible that the 2-wk-old B-line chicks were under a significant level of stress in the monsoon and summer seasons.

Neonates are generally vulnerable to cold stress, particularly in chickens, where it has been reported that the hypercorticosteronemic and hypothermic development are affected after postnatal hypothermic exposure [43]. In addition, the facultative thermogenesis of neonatal chicks, which functions through the uncoupling protein and mitochondrial substrate oxidation enzymes, appears to be unresponsive to corticotropin-releasing factor [44]. However, the apparent elevated stress levels detected in the 2-wk-old B-line chicks in this study were unlikely to be the effect of cold stress because significant differences were detected in the monsoon and summer seasons, but not in the winter season. Moreover, the ambient temperatures during the 2-wk brooding period were unlikely to be a significant contributing stress factor because they were controlled at or near the optimal level by the extra heat source provided (the 60-W bulb), in accordance with the general commercial broiler practice in Thailand. It is therefore more likely that the RH was a factor because it was extremely high in the summer and monsoon seasons, with daily maximum to minimum RH ranges recorded during the first 2-wk period of 75–90% to 50–65% and 80–100% to 60–80%, respectively, compared with 70–85% to 41–57% for the winter season (Figure 2). Thus, the RH

### Table 1. Effect of breed and season on the heterophil-to-lymphocyte (H:L) cell ratio of 3 breeds of chickens

<table>
<thead>
<tr>
<th>Breed (line)</th>
<th>Season</th>
<th>2 wk of age</th>
<th>4 wk of age</th>
<th>6 wk of age</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treatment effect</td>
<td>Broiler (B)</td>
<td>0.58 ± 0.04&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.80 ± 0.07&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.62 ± 0.06&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>Winter (W)</td>
<td>0.45 ± 0.04&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.66 ± 0.08&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.61 ± 0.07&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>Summer (S)</td>
<td>0.57 ± 0.05&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.68 ± 0.09&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.84 ± 0.04&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>Crossbred hybrid (C)</td>
<td>0.45 ± 0.03&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.34 ± 0.03&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.34 ± 0.01&lt;sup&gt;bc&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>Winter (W)</td>
<td>0.49 ± 0.06&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.40 ± 0.04&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.45 ± 0.05&lt;sup&gt;cd&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>Summer (S)</td>
<td>0.23 ± 0.03&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.18 ± 0.02&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.44 ± 0.03&lt;sup&gt;cd&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>Thai indigenous (T)</td>
<td>0.47 ± 0.03&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.32 ± 0.03&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>0.26 ± 0.02&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>Winter (W)</td>
<td>0.46 ± 0.04&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.34 ± 0.03&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.53 ± 0.03&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>Summer (S)</td>
<td>0.22 ± 0.03&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.18 ± 0.02&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.33 ± 0.02&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

| Breed effect     | Broiler (M + W + S) | 0.52 ± 0.02<sup>c</sup> | 0.72 ± 0.04<sup>ab</sup> | 0.69 ± 0.03<sup>d</sup> |
|                  | Crossbred (M + W + S) | 0.40 ± 0.03<sup>c</sup> | 0.31 ± 0.02<sup>c</sup> | 0.41 ± 0.02<sup>c</sup> |
|                  | Thai indigenous (M + W + S) | 0.39 ± 0.02<sup>c</sup> | 0.28 ± 0.02<sup>c</sup> | 0.37 ± 0.02<sup>c</sup> |

| Season effect    | Monsoon (B + C + T) | 0.50 ± 0.02<sup>c</sup> | 0.49 ± 0.04<sup>a</sup> | 0.40 ± 0.03<sup>c</sup> |
|                  | Winter (B + C + T)  | 0.47 ± 0.02<sup>c</sup> | 0.47 ± 0.03<sup>c</sup> | 0.53 ± 0.03<sup>c</sup> |
|                  | Summer (B + C + T)  | 0.34 ± 0.03<sup>c</sup> | 0.35 ± 0.04<sup>c</sup> | 0.54 ± 0.03<sup>c</sup> |

### Source of variation

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Breed</td>
<td>***</td>
</tr>
<tr>
<td>Season</td>
<td>***</td>
</tr>
<tr>
<td>Breed × season</td>
<td>***</td>
</tr>
</tbody>
</table>

**Means with different superscript letters within a column and section (treatment, breed, and season effect) are significantly different (P < 0.05).**

**P<0.001.**

**Data are shown as the mean ± 1 SEM.**
may have a significant role in stress induction in B-line chicks being brooded in the tropics. In line with this study, Lin et al. [38] reported that RH had a significant influence on the body temperature and thermal susceptibility of 1-wk-old broiler chicks.

It is interesting that when the H:L ratios of the 2-wk-old T- and C-line chicks were compared among the 3 seasons, the summer ratios were found to be significantly lower than the winter and monsoon ratios. If the RH was the only important influence on the stress levels of T- and C-line chicks, the winter RH should give the chicks the most comfort, whereas the monsoon RH should be the most stressful. However, contrary to this notion, we observed no significant difference in the stress response (H:L ratio) between the winter and monsoon seasons in this study. Thus, the RH was clearly less likely to be inducing a significant level of stress in the T and C chicken lines, which supports the above suggestion that the T- and C-line chicks are rather tolerant of a wide range of RH. The clearly reduced stress levels observed in the summer might then be the contribution of the summer temperature, which better maintained the brooding temperature at or near the optimum for the T- and C-line chicks. For this to be the case, given the use of 60-W heating lamps to maintain the temperature at the reported optimal developmental temperature for the B-line chicks, it suggests that a higher brooding temperature than the one recommended for commercial broilers may be favorable for the T- and C-line chicks, but this awaits confirmation.

A seasonal pattern in the H:L ratio similar to that seen in the 2-wk-old T- and C-line chicks was also observed in the 4-wk-old chicks except that, overall, their H:L ratios were lower than those of the 2-wk-old chicks. The stress levels observed in the winter and monsoon seasons were significantly higher than those observed in the summer, which emphasizes the fact that the T- and C-line chicks are rather susceptible to suboptimal temperatures during their first 4 wk of age. This was supported by behavioral observations, in which less movement was seen, with occasional huddling under the light bulb in the early morning in both groups. This raised an important concern regarding the application of the commercial broiler brooding practice, according to which the use of light bulbs to provide extra heat for the first 2 wk might not be sufficient for T- and C-line chicks, particularly in the winter and monsoon rearing periods, as mentioned above. Chicks from the T line have a relatively slow growth rate, with a BW approximately 4-to 5-fold lower than that of the fast-growing B-line chicks of the same age; therefore, the T-line chicks had a significantly higher body surface area-to-mass ratio. The body surface-to-mass ratio has been found to be a significant determinant of body heat loss [45], which could be the explanation for the cold stress, but not the heat stress, found in young T- and C-line chicks. In the natural habitat, the brooding of newborn chicks by mother hens is generally prolonged for at least 1 month. Both the brooding period and the temperature should therefore be studied further for their appropriate application in commercial flocks.

The H:L ratios determined in 4-wk-old B-line chickens were increased and were significantly higher than the levels expressed in T- and C-line chickens. In addition, the birds in group B were found panting and had decreased feed intake, particularly during the day; these birds were apparently experiencing a fairly high degree of heat stress [46]. Heat stress in commercial broilers has been demonstrated in many studies at ambient temperatures of higher than 30°C [22, 47, 48], which is comparable with the conditions represented in this study. In contrast to the findings in B-line chicks, the T- and C-line chicks of the same age seemed to be comfortable in the tropical warmth, which corresponded to the significantly lower H:L ratios we observed compared with those expressed in the B-line chicks. In the monsoon season, the stress response of B-line chicks was prominent, with significantly higher H:L ratios compared with those of B-line chicks reared in the winter and summer seasons. It is possible that the RH contributed significantly to tropical heat stress in the 4-wk-old B-line chicks. In accordance, a negative effect of RH on 4-wk-old broilers in environmental conditions comparable with the ones in this study was reported previously [37].

The heat stress in 6-wk-old B-line chickens appeared to be the most severe in the extremely hot and humid conditions of the summer (Figures 1 and 2), when the H:L ratio was found to
be significantly higher than those of the winter and monsoon seasons (Table 1). Daytime panting, feed deprivation, increased water consumption, watery excretion, and prostration were all observed in all the birds in group B. The heavy body mass of 6-wk-old B-line chickens could contribute a significant metabolic heat production load, as well as insulation (low relative surface area to mass), thereby superimposing the effect of the summer temperature in terms of the induced heat stress. The 6-wk-old T- and C-line chicks were found to express significantly lower stress levels in the monsoon and summer seasons. The elevated H:L ratios in both the T- and C-line chicks in the winter season were also higher than those in the 4-wk-old chicks.

**Innate Immune Response**

For AEC phagocytic activity, significant differences were observed because of the main effects (chicken breed and season) and their interaction (Table 2). As evaluated by the number of SRBC to phagocytic AEC, poor AEC phagocytic activity was found in the B-line chickens, with an activity approximately 1.5- and 1.7-fold lower with unopsinized and opsinized SRBC, respectively, compared with the C- and T-line birds of the corresponding age in the summer. This difference was, however, much less marked and was not statistically significant between the B-line chickens and the C- and T-line chickens in the winter season.

With respect to the percentage of phagocytic AEC for the unopsinized SRBC, a slightly different pattern was seen in that a significantly much lower level was found in the B group than was found in the corresponding T and C groups (2.6- and 2.9-fold, respectively) in the summer season, and that the level was still significantly lower (1.9-fold) in the B-line chickens than the T and C lines in the winter season. A similar trend

<table>
<thead>
<tr>
<th>Table 2. Effect of breed and season on the abdominal exudative cell (AEC) phagocytic activity of 3 breeds of chickens¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>Breed (line)</td>
</tr>
<tr>
<td>---------------</td>
</tr>
<tr>
<td>Broiler (B)</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>Crossbred hybrid (C)</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>Thai indigenous (T)</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>Breeding effect</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>Season effect</td>
</tr>
<tr>
<td></td>
</tr>
</tbody>
</table>

P-value

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>Breed</th>
<th>**</th>
<th>**</th>
<th>***</th>
<th>***</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Season</td>
<td>NS</td>
<td>***</td>
<td>***</td>
<td>***</td>
</tr>
<tr>
<td></td>
<td>Breed × season</td>
<td>*</td>
<td>**</td>
<td>***</td>
<td>***</td>
</tr>
</tbody>
</table>

1Data are shown as the mean ± 1 SEM. NT = not tested.

**P** < 0.05; **P** < 0.01; ***P** < 0.001; NS = P ≥ 0.05.
was seen with the phagocytosis of opsonized SRBC by AEC, with 2.2-fold and 1.2-fold lower percentages of phagocytic AEC being observed in the B-line chickens than in the corresponding T- and C-line chickens in the summer and winter seasons, respectively.

The differences in phagocytic activities of the AEC toward unopsonized SRBC between the winter and summer seasons were not significant in chickens of the B line but were significantly higher in the summer in the T- and C-line chicks. The number of unopsonized SRBC per phagocytic AEC was significantly higher in the summer than in the winter in all 3 chicken lines, whereas the phagocytic activity of the AEC for opsonized SRBC in the summer season was significantly higher than that in the winter season only in the T line. This expression of innate immunity was negatively related to the responsive stress (as mentioned previously), in which chickens from the B line appeared to be more stressed in both the winter and summer seasons, whereas those from the T and C lines were comparatively less stressed and seemed to be more comfortable in the summer compared with the winter. Therefore, the stress induced in response to the tropical environmental conditions might be a crucial factor contributing to the inferiority of the innate immunity in high-meat-yielding B-line chickens. Indeed, it has been reported that the AEC phagocytic activity of B-line chickens is negatively affected by heat stress and that this could be ameliorated by vitamin E [24] or selenium [49] treatment.

The adherent AEC elicited by Sephadex G-50 were found to consist mostly of macrophages, the major cells of the innate immune system, and principal antigen-presenting cells. Thus, this study revealed a likely significant reduction in the innate immune system function in chicks of the high-meat-yielding B line compared with the low- and moderate-meat-yielding T and C chicken lines. Such a decline in the innate immunity as a trade-off for increased meat production has been reported previously [23].

Humoral Immune Response

The humoral immune responses were evaluated in terms of the MES and MER titers against Newcastle vaccine, which correspond to the IgM and IgG titers, respectively [35]. Both the MES and MER titers at 7 d after the booster vaccine were highly significantly affected by the season, but only the MER was affected by the chicken breed, whereas significant interactions between chicken breed and season were noted for both the MER and MES (Table 3). It is interesting that the MES titers expressed in the chickens from the T line were significantly lower in the winter season and higher in the summer season compared with the other 2 corresponding chicken lines (B and C). These IgM responses appeared to correlate with the stress responses of the T-line chickens, in which the stress levels were higher in the winter, and therefore could reflect the better adaptation to ambient heat of the T-line chickens.

The MES titers of all groups decreased at 14 d after the booster vaccine, and chickens from the C and T lines had significantly lower titers than those observed in the B-line chickens. The IgM titers of the C- and T-line chickens appeared to decline sooner than those of the B-line chickens. However, the MER titers expressed in the B-line chickens were significantly lower than those in the other 2 lines at both 7 and 14 d postvaccination in all seasons. The B-line chickens appeared to have a lower secondary (IgG antibody) immune response compared with chickens from the T and C lines. Thus, the C and T chicken lines are potentially superior to the B-line chickens in terms of humoral immune function, and the impact of the tropical climate-induced stress may aggravate the humoral immune responses of B-line chickens. In accordance with this study, the effect of heat stress on the suppression of the humoral immune response has been reported previously [25, 50]. However, it has been reported that the cell-mediated response, but not the humoral immune response, is either enhanced [51, 52] or impaired [27, 39] by chronic heat or cold stress. This may mean that T-helper 2 and B-lymphocyte cells may not be affected. Such contrasting findings could be due to the different stress-induction protocols applied in each experiment. It is therefore essential to explore the effect of environmental conditions that are closer to (ideally, identical to) the actual rearing conditions for the valid applicability of the research findings.

The negative effect on AEC or macrophage phagocytic activity mentioned above could be a
factor contributing to the low antibody titer of the heat-stressed B-line chickens because AEC have an essential role in antigen presentation, an essential step in initiating an acquired immune response [53]. Therefore, stress induced by the tropical environmental conditions could have deleterious effects on the innate and humoral immunity, and these may depend on the genetic background of the chicken.

**CONCLUSIONS AND APPLICATIONS**

1. Under tropical rearing conditions, Thai indigenous (T-line) chickens and Thai indigenous crossbred (C-line) chickens generally expressed significantly lower stress levels than commercial broiler (B-line) chickens in all seasons.

2. The T- and C-line chickens possessed an apparently stronger innate and humoral immunity than the B-line chickens.

3. These findings could be applied for further improvement of a commercial meat-type chicken to be reared in tropical climate conditions.

**REFERENCES AND NOTES**


32. Sephadex G-50, Sigma-Aldrich Corp., St. Louis, MO.

33. Complete medium [Iscove's Modified Dulbecco's Medium and 5% (vol/vol) fetal bovine serum], Gibco, Carlsbad, CA.

34. Organic Permount, Fisher Scientific, Waltham, MA.


at different ambient temperatures. I. One week of age. Poult. Sci. 84:1166–1172.


Acknowledgments

This study was supported by a grant from the Thailand Research Fund (TRF), Bangkok.