Effect of heat and several additives related to stress levels on fluctuating asymmetry, heterophil:lymphocyte ratio, and tonic immobility duration in White Leghorn chicks

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ABSTRACT The purpose of this study was to analyze the effects of heat and several additives related to stress on fluctuating asymmetry (groups 1 to 10), heterophil:lymphocyte ratio (groups 1 to 3 and 8 to 10), and tonic immobility duration (groups 1 to 7 and 10) in White Leghorn chicks at 42 d of age. Chicks in group 1 (heat) were reared with temperatures 8°C greater than those of the control group. Groups 2 to 9 consisted of chicks reared with temperatures 8°C greater than those of the control group and addition of capsaicin, allicin, ascorbic acid, tryptophan, brewer’s yeast, lactic acid, corticosterone, or cholesterol in diet. Chicks in group 10 (control) were reared with standard temperatures. Heat effect was significant (P < 0.05) for the heterophil:lymphocyte ratio, which was greater in heat-stressed chicks without any additives and smaller in control chicks. There were no significant differences for the fluctuating asymmetry and the tonic immobility duration between both groups. Heterophil:lymphocyte ratio for heat-stressed chicks with capsaicin or allicin was significantly lower (P < 0.05) than that of heat-stressed chicks without any additives. Capsaicin effect was not significant for the fluctuating asymmetry and the tonic immobility duration, whereas allicin significantly increased fluctuating asymmetry of wing length and tonic immobility duration (P < 0.05). The addition of lactic acid or corticosterone resulted in greater fluctuating asymmetry of wing length of heat-stressed chicks (P < 0.05). In conclusion, an increased heterophil:lymphocyte ratio was found in heat-stressed chicks without additives, indicating that it is a more reliable indicator of the effect of heat in chicks. In addition, dietary capsaicin or allicin supplementation was effective to alleviate the stress induced by the high temperature, as indicated by a lower heterophil:lymphocyte ratio.

Key words: heat, additive, well-being, stress, fear

INTRODUCTION

High temperature imposes severe stress on birds and leads to important economic losses in the poultry industry. Although birds perform well within a relatively wide range of temperatures, between 10 and 27°C (Daghir, 2009), temperatures above 30°C may cause stress in adult hens (Daghir, 1995) and broiler chickens (Geraert et al., 1996a).

Fluctuating asymmetry (FA; small random deviations from right-left symmetry in bilateral traits) has been reported to reflect well-being status and chronic stress (Møller and Swaddle, 1997), and the effect of heat on the FA has been studied only in broiler chickens by Yalcin et al. (2003). Developmental stability, estimated by FA, serves for assessing the degree to which an individual is able to buffer its development when it is stressed. Yalcin et al. (2003) investigated the effect of heat (32 to 35°C) on relative asymmetry of shank length, shank width, and face length. The effect of heat on the heterophil:lymphocyte ratio (a reliable indicator of stress; Gross and Siegel, 1983; Davis et al., 2008) has been analyzed in several experiments using broiler chickens (McFarlane and Curtis, 1989; Zulkifli et al., 1999, 2009; Altan et al., 2003; Yalcin et al., 2003; Akşit et al., 2006). The effect of heat on the tonic immobility duration (a traditional measure of stress in poultry; Gallup, 1979) has been analyzed in 4 experiments with broilers (Zulkifli et al., 1999; Altan et al., 2003; Yalcin et al., 2003; Akşit et al., 2006).

Several feed compounds are related to stress levels in animals and some may be used for preventing heat effect. Between them are some antioxidants: capsaicin (Lee et al., 2003), allicin (Yin and Cheng, 1998), and ascorbic acid (Whitehead and Keller, 2003), because
the oxidative injury induced by high ambient temperatures has been demonstrated in several studies (Altan et al., 2003; Lin et al., 2006; Mujahid et al., 2006), and the oxidative stress should be considered as part of the stress response of chickens to heat exposure. In Japanese quails, Ipek et al. (2007) found that ascorbic acid supplementation decreased the heterophil:lymphocyte ratio and was adequate to alleviate heat effect, although Campo and Dávila (2002) did not find significant effects of dietary ascorbic acid supplementation on the heterophil:lymphocyte ratio in heat-stressed hens. The other 2 additives, tryptophan (a precursor of niacin, another antioxidant vitamin; Shea-Moore et al., 1996) and brewer’s yeast (also with antioxidant properties; Zhang et al., 2005), might be used to prevent heat effects. Campo and Dávila (2002) did not find significant effect of dietary tryptophan or brewer’s yeast supplementation on the heterophil:lymphocyte ratio of heat-stressed hens.

On the other hand, several compounds, lactic acid, corticosterone, and cholesterol (a corticosterone precursor), increase in stress situations (Geraert et al., 1996b; Imaeda, 2000; Debout et al., 2005) and can be used to study the effects of stress. Oral corticosterone administration increased heterophil:lymphocyte ratio in broiler chickens (Post et al., 2003) and laying hens (Shini et al., 2009), and prenatal exposure to corticosterone resulted in greater FA of tarsus length (Eriksen et al., 2003). Campo and Dávila (2002) evaluated the effect of dietary supplementation with lactic acid on the heterophil:lymphocyte ratio in heat-stressed hens; the ratio increased significantly in hens exposed to heat and lactic acid supplementation.

We predicted that heat-stressed birds would show more asymmetrical morphological traits, a longer duration of tonic immobility, and increased heterophil:lymphocyte ratio and that these effects might be corrected or increased with different additives. Relative to the existing literature, the study complements with layers the only previous experiment to analyze the effect of heat on the FA with meat animals and the experiments analyzing the effect of heat on the heterophil:lymphocyte ratio of broilers. Additionally, it complements the contradictory results that have been obtained for the duration of tonic immobility in broilers. As far we know, the effects of several feed additives on the FA, heterophil:lymphocyte ratio, and tonic immobility duration of heat-stressed chicks have not been studied yet. The objective of this study was to evaluate the effect of heat and several additives related to stress levels, supplemental capsaicin, allicin, ascorbic acid, tryptophan, brewer’s yeast, lactic acid, corticosterone, and cholesterol in diets, on FA in White Leghorn chicks. Additionally, the effects of heat on heterophil:lymphocyte ratio (with or without capsaicin, allicin, corticosterone, and cholesterol) and tonic immobility duration (with or without capsaicin, allicin, ascorbic acid, tryptophan, brewer’s yeast, and lactic acid) were also analyzed.

MATERIALS AND METHODS

Birds and Experimental Design

The data were obtained from chicks of a White Leghorn population originated by crossing 3 strains selected for egg number and egg weight (Campo and Jurado, 1982). A stress of temperatures 8°C greater than those in the control group from 1 to 42 d of age and different feed compounds were used: capsaicin supplemented at 50 mg/kg; allicin supplemented at 0.2 g/kg, ascorbic acid supplemented at 1 g/kg, tryptophan supplemented at 5 g/kg, brewer’s yeast supplemented at 0.5%, lactic acid supplemented at 20 g/kg, corticosterone supplemented at 60 mg/kg, and cholesterol supplemented at 6 g/kg. Control chicks were reared under standard temperatures controlled with electric heaters (33 to 35°C at the chick level during the first week followed by a reduction of 3°C each week until the temperature was 18 to 20°C at the sixth week of age). Products were bought from Sigma-Aldrich Chemical Co. (St. Louis, MO). They were added to the feed except allicin and lactic acid, which were added to the water. Chicks were reared in cages at a density of 10 birds/m². Artificial light was provided during the first week (23L:1D). Chicks were fed standard rearing diets without any prophylactic antibiotics, containing 19% CP, 2,800 kcal ME/kg, 1% Ca, 0.5% available P, and 12 mg/kg of ascorbic acid and based on the Spanish standard for White Leghorns (approximately 1 kg at 42 d of age per chick; i.e., 10 g/d, wk 1; 16 g/d, wk 2; 22 g/d, wk 3; 28 g/d, wk 4; 34 g/d, wk 5; and 40 g/d, wk 6, respectively). Water was supplied as follows (per 20 chicks): 0.4 L/d, wk 1; 0.7 L/d, wk 2; 1 L/d, wk 3; 1.3 L/d, wk 4; 1.6 L/d, wk 5; and 1.9 L/d, wk 6, respectively. Chicks consumed all feed and water.

A total of 440 chicks from 2 different replicates (hatches) separated by 14 d were sampled. There were 2 cages (20 chicks per cage) in each treatment group (1 cage per replicate) and 4 cages (20 chicks per cage) in the control group (2 cages per replicate). Ten groups were used. Group 1 (heat) consisted of 40 chicks (20 in each replicate) reared with temperatures 8°C greater than those of the control group. Group 2 (heat + capsaicin) consisted of 40 additional chicks (20 in each replicate) reared with temperatures 8°C greater than those of the control group and the addition of capsaicin. Group 3 (heat + allicin) consisted of 40 additional chicks (20 in each replicate) reared with temperatures 8°C greater than those of the control group and the addition of allicin. Group 4 (heat + ascorbic acid) consisted of 40 additional chicks (20 in each replicate) reared with temperatures 8°C greater than those of the control group and the addition of ascorbic acid. Group 5 (heat + tryptophan) consisted of 40 additional chicks (20 in each replicate) reared with temperatures 8°C greater than those of the control group and the addition of tryptophan. Group 6 (heat + brewer’s yeast) consisted...
of 40 additional chicks (20 in each replicate) reared with temperatures 8°C greater than those of the control group and the addition of brewer’s yeast. Group 7 (heat + lactic acid) consisted of 40 additional chicks (20 in each replicate) reared with temperatures 8°C greater than those of the control group and the addition of lactic acid. Group 8 (heat + corticosterone) consisted of 40 additional chicks (20 in each replicate) reared with temperatures 8°C greater than those of the control group and the addition of corticosterone. Group 9 (heat + cholesterol) consisted of 40 additional chicks (20 in each replicate) reared with temperatures 8°C greater than those of the control group and the addition of cholesterol. Finally, group 10 (control) consisted of 80 additional chicks (40 in each replicate) reared with standard temperatures. The experimental protocol was approved by the ethical commission of the Instituto Nacional de Investigación y Tecnología Agraria y Alimentaria.

**Measurement of FA (10 Groups)**

The morphological traits measured were both right (R) and left (L) middle toe length (from the metatarsus to the nail), leg (metatarsus) length (from the hock joint to the middle toe), wing (radius) length (from the humerus joint to the carpus), and leg width (in the hock joint), in all live birds at 42 d of age. Right and left values of a bird were taken during the same session. All 3 lengths and leg width were measured in millimeters using a digital caliper. Trait size was the mean of the right and left traits \([\frac{(R + L)}{2}]\). All traits showed normal frequency distributions. The FA for a trait was defined by the absolute difference between sides \([|R - L|]\). A series of steps (Palmer, 1994; Knierim et al., 2007) was followed before identifying exhibited asymmetry as FA (normal distribution of signed right minus left differences with a mean of zero) because there are several confounding factors that complicate the analysis of asymmetry (details can be found in the preliminary study by Campo et al., 2008). First, the presence of antisymmetry (AS; nonnormal distribution with a mean of zero) was tested by inspecting the distribution of \((R - L)\). Departures from normality were assessed using kurtosis measures; AS is characterized by bimodal (or broad-peaked) distributions, tending to be platykurtic (more intermediate values than the normal distribution). Second, FA and measurement error are normally distributed over a mean of zero. Thus, it is essential to show that the variance in asymmetry observed between individuals is greater than the variance due to measurement error; the FA is often small and sometimes of the same magnitude as measurement error. The effect of measurement error was calculated from a subsample of 20 birds randomly selected and measured 3 times on 3 different days. These repeated measurements were analyzed using a 2-way ANOVA (Leamy, 1984) with side (fixed) and bird (random) as main factors (1 and 19 df), their interaction (19 df), and the measurement error (80 df). Significant variation between sides indicates directional asymmetry (DA; normal distribution with a nonzero mean), whereas a significant interaction indicates significant FA (in the absence of AS). In the preliminary study by Campo et al. (2008), toe, leg, and wing lengths were considered suitable for the study of FA based on the presence of a high FA-to-measurement error and the absence of DA and AS. Leg width exhibited a significant level of FA and DA; it was included in the study by subtracting the mean of \((R - L)\), a measure of DA, from each value of FA to eliminate DA (van Valen, 1962). Finally, the product-moment correlation between FA and trait value was used to determine if they were independent. If a positive relationship was found between the mean value and the asymmetry of a trait, this effect was removed by dividing the absolute asymmetry score by the trait mean, defined as the relative FA: \([2|\frac{R - L}{(R + L)}|]\). Relative FA was used for all traits; it had distributions that were not normal and were transformed to arcsin square root before analysis. As an alternative to the analysis of FA based on the 4 single morphological traits, combined relative asymmetry was considered (defined as the mean of the relative asymmetries of the different traits); the analysis based on the combined FA may be a more reliable indicator of stress (Leung et al., 2000).

**Measurement of Heterophil:Lymphocyte Ratio (6 Groups)**

To obtain the heterophil:lymphocyte ratio (on the same day and before the FA measures), all chicks of groups 1 to 3 and 8 to 10 were caught randomly and carried to a separate room. Heterophil:lymphocyte ratio was not measured with supplemental ascorbic acid, tryptophan, brewer’s yeast, and lactic acid because it had been previously analyzed by Campo and Dávila (2002). Blood was collected immediately, taking 2 drops of blood from a small puncture in the comb of each chick, 1 drop being smeared on each of 2 glass slides. The smears were stained using May-Grünwald and Giemsa stains (Lucas and Jamroz, 1961), approximately 2 to 4 h after methyl alcohol fixation. One hundred leukocytes, including granular (heterophils, eosinophils, and basophils) and nongranular (lymphocytes and monocytes), were counted on 1 slide of each chick (the other slide was supplementary), and the heterophil:lymphocyte ratio was calculated. Ratios were transformed to square root before analysis.

**Measurement of Tonic Immobility Duration (8 Groups)**

All chicks of groups 1 to 7 and 10 were tested for tonic immobility on the day after the blood sampling and the FA measures. Duration of tonic immobility was not measured with supplemental corticosterone and
choline because it had been previously analyzed by Campo and Carnicer (1994, 1995). Chicks were caught randomly and carried in an upright position to a separate neighboring room. A few seconds after the chick was caught, tonic immobility was induced by placing the bird on its back with the head hanging in a U-shaped wooden cradle (Jones and Faure, 1981). The chick was restrained for 10 s. The observer sat in full view of the chick, about 1 m away, and fixed his eyes on the chick to give the fear-inducing properties of eye contact. If the chick remained immobile for 10 s after the experimenter removed his hands, a stopwatch was started to record latency (s) until the chick righted itself. If the chick righted itself in less than 10 s, then it was considered that tonic immobility had not been induced, and the restraint procedure was repeated (3 times maximum). If the chick did not show a righting response over the 10-min test period, a maximum score of 600 s was given for righting time. Thus, tonic immobility duration ranged from 0 to 600 s. Durations were logarithmically transformed before analysis.

**Statistical Analysis**

To test the differences in FA, heterophil:lymphocyte ratio, and tonic immobility duration between groups of chicks, a 2-way ANOVA (Sokal and Rohlf, 1981) was used with the statistical model $x_{ijk} = \mu + G_i + r_j + Grij + \varepsilon_{ijk}$, where $x_{ijk}$ = the analyzed measurement; $\mu$ = the overall mean; $G_i$ = the effect of group ($i = 1 \ldots 10$, $i = 1 \ldots 6$, or $i = 1 \ldots 8$); $r_j$ = the effect of replicate ($j = 1 \ldots 2$); $Grij$ = the interaction (confounded with the effect of cage); and $\varepsilon_{ijk}$ = the residual ($k = 1 \ldots 20$ or $k = 1 \ldots 40$). The experimental unit was the chick. Group was considered the fixed effect and replicates were assumed to be a random effect. There were no significant differences between replicates or its interaction (cage), and they were pooled with the residual to give a 1-way model of group effect ($x_{ij} = \mu + G_i + \varepsilon_{ij}$). The significance of differences among group means was evaluated using the Student-Newman-Keuls multiple range test (Snedecor and Cochran, 1980). The SAS statistical package, GLM procedure, was used for data analysis.

### RESULTS

Mean values indicating the effect of heat, capsaicin, allicin, ascorbic acid, tryptophan, brewer’s yeast, lactic acid, corticosterone, and cholesterol on FA are summarized in Table 1 (with the final number of chicks in each group). Mortality was similar in the heat and control groups (5 and 6%, respectively) and greater in the capsaicin, lactic acid, and corticosterone groups (27, 25, and 32%, respectively). There were significant differences between groups for the relative asymmetry of wing length ($P < 0.001$). The relative asymmetry of wing length of heat-stressed chicks without any additives did not differ significantly from that of control birds, whereas the relative asymmetry of wing length of heat-stressed chicks with allicin, lactic acid, or corticosterone was significantly higher than that of heat-stressed chicks without any additives. There were no significant differences between groups for the relative asymmetry of toe length, leg length, leg width, and the combined relative asymmetry of the 4 traits.

Mean values indicating the effect of heat, capsaicin, allicin, corticosterone, and cholesterol on heterophil:lymphocyte ratio are indicated in Table 2. There were significant differences between groups ($P < 0.001$) for the heterophil:lymphocyte ratio. Heterophil:lymphocyte ratio was significantly higher in heat-stressed chicks without any additives than in control chicks, the former having significant heterophilia and lymphopenia. Capsaicin and allicin had a significant effect, with heterophil:lymphocyte ratio (and heterophil number) of heat-stressed chicks with one of these additives being significantly lower than that of heat-stressed chicks without any additives (with the opposite being true for lymphocyte number).

Mean values indicating the effect of heat, capsaicin, allicin, ascorbic acid, tryptophan, brewer’s yeast, and lactic acid on tonic immobility duration are included in Table 2. There were significant differences between groups ($P < 0.01$) for duration of tonic immobility. The tonic immobility of heat-stressed chicks without any additives was similar to that of control chicks, whereas the tonic immobility of heat-stressed chicks with allicin, ascorbic acid, tryptophan, brewer’s yeast, lactic acid, corticosterone, and cholesterol was significantly lower than that of control chicks.

### Table 1. Mean relative asymmetry (×100) of various morphological traits in 10 different groups of chicks ($n = 38, 29, 33, 33, 35, 37, 30, 27, 39$, and $75$, respectively)

<table>
<thead>
<tr>
<th>Group</th>
<th>Toe length</th>
<th>Leg length</th>
<th>Wing length</th>
<th>Leg width</th>
<th>Combined</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heat</td>
<td>2.44</td>
<td>2.48</td>
<td>1.63c</td>
<td>4.79</td>
<td>2.84</td>
</tr>
<tr>
<td>Heat + capsaicin</td>
<td>3.10</td>
<td>5.67</td>
<td>1.55c</td>
<td>5.01</td>
<td>3.83</td>
</tr>
<tr>
<td>Heat + allicin</td>
<td>2.70</td>
<td>2.09</td>
<td>2.06bc</td>
<td>4.49</td>
<td>2.83</td>
</tr>
<tr>
<td>Heat + ascorbic acid</td>
<td>2.74</td>
<td>1.90</td>
<td>1.82c</td>
<td>5.09</td>
<td>2.89</td>
</tr>
<tr>
<td>Heat + tryptophan</td>
<td>2.38</td>
<td>2.26</td>
<td>2.38bc</td>
<td>5.14</td>
<td>3.04</td>
</tr>
<tr>
<td>Heat + brewer’s yeast</td>
<td>2.45</td>
<td>3.43</td>
<td>4.36a</td>
<td>5.33</td>
<td>3.89</td>
</tr>
<tr>
<td>Heat + lactic acid</td>
<td>3.14</td>
<td>2.79</td>
<td>3.24ab</td>
<td>5.79</td>
<td>3.74</td>
</tr>
<tr>
<td>Heat + corticosterone</td>
<td>3.37</td>
<td>2.70</td>
<td>1.62c</td>
<td>4.43</td>
<td>3.03</td>
</tr>
<tr>
<td>Heat + cholesterol</td>
<td>2.71</td>
<td>2.49</td>
<td>1.70c</td>
<td>4.86</td>
<td>2.95</td>
</tr>
<tr>
<td>Control</td>
<td>5.38</td>
<td>2.84</td>
<td>2.54</td>
<td>17.49</td>
<td>2.24</td>
</tr>
</tbody>
</table>

* Means within the same trait with no common superscript differ ($P < 0.05$).
Table 2. Mean heterophil number, lymphocyte number, and heterophil:lymphocyte ratio\(^1\) in 6 different groups of chicks (\(n = 38, 29, 33, 27, 39,\) and 75, respectively) and tonic immobility duration (s)\(^2\) in 8 different groups of chicks (\(n = 38, 29, 33, 35, 37, 30,\) and 75, respectively)

<table>
<thead>
<tr>
<th>Group</th>
<th>Heterophil number</th>
<th>Lymphocyte number</th>
<th>Heterophil:lymphocyte</th>
<th>Tonic immobility</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heat</td>
<td>35(^a)</td>
<td>63(^b)</td>
<td>0.58(^a)</td>
<td>90(^b)</td>
</tr>
<tr>
<td>Heat + capsaicin</td>
<td>27(^b)</td>
<td>71(^a)</td>
<td>0.38(^b)</td>
<td>95(^b)</td>
</tr>
<tr>
<td>Heat + allicin</td>
<td>25(^b)</td>
<td>73(^a)</td>
<td>0.36(^b)</td>
<td>215(^a)</td>
</tr>
<tr>
<td>Heat + ascorbic acid</td>
<td>34(^a)</td>
<td>64(^b)</td>
<td>0.58(^a)</td>
<td>106(^b)</td>
</tr>
<tr>
<td>Heat + tryptophan</td>
<td>36(^a)</td>
<td>62(^b)</td>
<td>0.65(^b)</td>
<td>129(^b)</td>
</tr>
<tr>
<td>Heat + brewer’s yeast</td>
<td>34(^a)</td>
<td>64(^b)</td>
<td>0.58(^a)</td>
<td>151(^b)</td>
</tr>
<tr>
<td>Heat + lactic acid</td>
<td>129(^b)</td>
<td>106(^b)</td>
<td>0.34(^b)</td>
<td>99(^b)</td>
</tr>
<tr>
<td>Heat + corticosterone</td>
<td>76(^c)</td>
<td>75(^c)</td>
<td>0.04(^c)</td>
<td>9.649(^c)</td>
</tr>
<tr>
<td>Error mean square</td>
<td>76</td>
<td>75</td>
<td>0.04</td>
<td></td>
</tr>
</tbody>
</table>

\(^{a,b}\)Means within the same trait with no common superscript differ (\(P < 0.05\)).

\(^1\)Heterophil number, lymphocyte number, and heterophil:lymphocyte ratio were not measured with supplemental ascorbic acid, tryptophan, brewer’s yeast, and lactic acid.

\(^2\)Tonic immobility duration was not measured with supplemental corticosterone and cholesterol.

cin was significantly greater than that of heat-stressed chicks without any additives.

**DISCUSSION**

We did not find significant association between heat (temperatures 8°C greater than those of the control group between 1 and 42 d of age) and FA because FA for toe length, leg length, wing length, and leg width was similar in heat-stressed chicks without any additives and control chicks, suggesting that it does not constitute a sensitive indicator of thermal stress effect in chicks. This result is consistent with those found by Yalcin et al. (2003), who did not find significant differences between heat-stressed (temperatures 10°C greater than those of the control group between 1000 and 1700 h each day from 21 to 42 d of age) and control broiler chicks in relative asymmetry of leg length, leg width, and face length. Alternatively, difference in combined FA of toe length, leg length, wing length, and leg width was not significant either. In disagreement with this result, Yalcin et al. (2003) indicated that heat exposure resulted in higher combined relative asymmetry of leg length, leg width, and face length. However, these authors used a nonparametric method (Kruskal-Wallis), with a parametric test (ANOVA) always being preferred to assess the effect of treatments on FA (Gangestad and Thornhill, 1998). Although the weight of the chicks was not measured in our study, Yalcin et al. (2003) found that the lowest BW at 42 d was for heat-stressed broilers and heaviest for the controls, the effect on grow also being considered an indicator of well-being. Heat-stressed chicks without any additives had the heterophil:lymphocyte ratio significantly higher, almost 70%, than control chicks. This confirmed earlier reports by McFarlane and Curtis (1989), Zulkifli et al. (1999, 2009), Altan et al. (2003), Yalcin et al. (2003), and Akşit et al. (2006) that heat challenge may elevate heterophil:lymphocyte ratio in broiler chickens; in these studies, the heat exposure was very heterogeneous, ranging from 38°C for 3 h at 36 d of age to 34°C for 21 d at 21 d of age. In the present study, the heterophil:lymphocyte ratio was 0.58 for the heat-stressed chicks without any additives. This indicates a moderate effect of high temperature, bearing in mind that 0.2, 0.5, and 0.8 for the heterophil:lymphocyte ratio are characteristic of low, optimal, and high degrees of stress, respectively (Gross and Siegel, 1993). Duration of tonic immobility was similar in heat-stressed chicks without any additives and control chicks, suggesting that heat-stressed chicks did not tend to be more fearful than control chicks and that response to a thermal stressor is not associated with fear-related behavior. This result is in agreement with those found by Zulkifli et al. (1999) and Akşit et al. (2006) indicating that tonic immobility duration was not affected by heat in broiler chickens, although it is in disagreement with the increases found by Altan et al. (2003) and Yalcin et al. (2003) in broiler chickens. Variation in genetic background and heat exposure could be responsible for the discrepancies in the results obtained.

Supplementation of capsaicin or allicin significantly decreased heterophil:lymphocyte ratio for heat-stressed chicks with one of these additives in comparison with that for heat-stressed chicks without any additives (66 and 62%, respectively), suggesting that they have a positive effect to alleviate the effects of heat. Heat-stressed chicks with capsaicin or allicin had a significantly lower heterophil number (heteropenia) and higher lymphocyte number (lymphophilia) than heat-stressed chicks without any additives. In this way, the oxidative injury induced by high ambient temperatures (Altan et al., 2003; Lin et al., 2006; Mujahid et al., 2006) could be reduced by the action of some antioxidants like capsaicin and allicin, and nutritional strategies aimed to alleviate the negative effects of heat by supplementing some additives could be advantageous. However, the high mortality observed in the capsaicin group of chicks, as high as those in the lactic acid and corticosterone groups, suggests a serious limitation for the application of this
compound. Mortality can be considered as the final indicator of well-being, although it could be argued that our sample size was not enough to give a representative view of mortality because we chose a large number of groups rather than large numbers of chicks in each group. Capsaicin is not an irritant for wild birds (Tewksbury and Nabhan, 2001), the seeds of Capsicum being predominantly dispersed by birds, but domestic chicken might be affected by capsaicin. The FA of wing length and the tonic immobility duration were significantly greater for heat-stressed chicks with allicin than for heat-stressed chicks without any additives, suggesting that the addition of allicin had different effects depending on the stress indicator that was measured. Addition of ascorbic acid, tryptophan, or brewer’s yeast did not significantly change measurements for FA and tonic immobility duration because these values were similar in heat-stressed chicks with one of these additives and in heat-stressed chicks without any additives. The results agree with those reporting no significant effect of dietary tryptophan (supplemented at 2 to 6 g/kg) on tonic immobility duration of broiler chickens reared under normal temperatures (MacKenzie et al., 2004; Corzo et al., 2005). Although the heterophil:lymphocyte ratio was not measured in these 3 groups of chicks, Campo and Dávila (2002) did not find significant effects of ascorbic acid, tryptophan, or brewer’s yeast supplementation on the heterophil:lymphocyte ratio of heat-stressed chickens. Results are in disagreement with those by Ipek et al. (2007) in Japanese quails and Mahmoud et al. (2004) in broiler chickens, who reported that dietary ascorbic acid (supplemented at 0.5 g/kg) decreased heterophil:lymphocyte ratio and plasma corticosterone response under high ambient temperature. The addition of one of these compounds might have different effects depending on the poultry species that was used and the stress indicator that was measured.

The addition of lactic acid to the diet of heat-stressed chicks significantly increased the FA of wing length. Heat-stressed chicks with this additive had significantly greater FA of wing length (more than 2.5×) than heat-stressed chicks without lactic acid. Although the heterophil:lymphocyte ratio was not measured in this group of chicks, Campo and Dávila (2002) found that heat-stressed chickens with lactic acid supplementation had higher heterophil:lymphocyte ratios than those of the heat-stressed chickens without lactic acid, indicating the effect of 2 concurrent stressors (heat and supplemental lactic acid) in the chicks. Contrary to what would be expected, supplemental corticosterone or cholesterol in the diet of heat-stressed chicks did not significantly change the heterophil:lymphocyte ratio, heat-stressed chicks with one of these additives or without any additives having similar values, although corticosterone supplementation to diet significantly increased the FA of wing length.

In conclusion, although an elevated FA or tonic immobility duration was not detected in heat-stressed chicks without any additives, an increased heterophil:lymphocyte ratio was apparent, indicating that the heterophil:lymphocyte ratio is a more reliable indicator of the effects of heat in chicks. The degree of stress experienced by chicks under high temperature conditions can be lesser than that by adult birds. The addition of capsaicin significantly decreased the heterophil:lymphocyte ratio, suggesting that it is effective to alleviate the stress induced by the high temperature. Effect of allicin was not consistent because it resulted in a smaller heterophil:lymphocyte ratio and greater FA of wing length and duration of tonic immobility. Lactic acid and corticosterone resulted in greater FA of wing length.

REFERENCES


HEAT, DIETARY ADDITIVES, AND STRESS


