ENVIRONMENT, WELL-BEING, AND BEHAVIOR

Genetic variations alter production and behavioral responses following heat stress in 2 strains of laying hens

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ABSTRACT Genetic differences alter the type and degree of hens’ responses and their ability to adapt to a stressor. This study examined the effects of genotypic variations on the productivity and behavior of laying hens following heat stress (HS). Two strains of White Leghorn hens were used: DXL (Dekalb XL), a commercial strain individually selected for egg production and KGB (kind, gentle bird), a strain selected for high group productivity and survivability. Ninety hens (48 DXL and 42 KGB) at 28 wk of age were randomly assigned to either a hot (H: mean = 32.6°C) or control (C: mean = 24.3°C) treatment and housed in pairs by strain for 9 d. Egg production and quality, behavior, body and organ weights, and circulating hormone concentrations were measured. Heat-stressed hens had lower egg production [adjusted (adj) \( P < 0.001 \)] than their respective controls. Among H-DXL hens, egg weight tended to be reduced at d 1 and was reduced at d 9 (adj \( P = 0.007 \)), but was reduced only at d 9 among H-KGB hens (adj \( P = 0.007 \)). Eggshell thickness was also reduced among H hens at d 9 (adj \( P = 0.007 \)), especially among H-KGB hens (adj \( P = 0.01 \)). Plasma triiodothyronine concentration was reduced among H-hens (adj \( P = 0.01 \)), especially among H-DXL hens (adj \( P = 0.01 \)). Neither temperature nor strain affected the plasma thyroxine and plasma and yolk corticosterone concentrations. Heat-stressed hens spent less time walking (adj \( P = 0.001 \)) and more time drinking (adj \( P = 0.007 \)) and resting (adj \( P = 0.001 \)) than C-hens. The results indicate that although HS reduced production and caused behavioral changes among hens from both strains, the responses differed by genotype. The data provide evidence that genetic selection is a useful strategy for reducing HS response in laying hens. The results provide insights for conducting future studies to develop heat-resistant strains to improve hen well-being, especially under the current commercial conditions.

Key words: behavior, heat stress, laying hen, physiology, production

INTRODUCTION

High ambient temperatures result in economic loss and reduced bird welfare in the poultry industry. Elevated temperatures can alter the activity of the endocrine system of chickens, which may result in changes such as increased plasma corticosterone concentration, a commonly used biomarker indicative of certain types of stress (Star et al., 2008a; Quinteiro-Filho et al., 2010). Unbalanced endocrine homeostasis directly and indirectly affects hens’ reproduction, reducing both egg production and egg quality, including egg weight and eggshell thickness (Lin et al., 2004; Mashaly et al., 2004; Franco-Jimenez et al., 2007). Elevated temperatures also increase mortality in both layers (Mashaly et al., 2004) and broilers (Quinteiro-Filho et al., 2010). Seemingly, the poultry industry will face a greater problem associated with heat stress (HS) in the near future, as the continuously changing global climate lengthens the hot season and increases the geographic areas affected by high environmental temperatures (Battisti and Naylor, 2009; Hansen et al., 2010).

Reduced egg quality and production are largely mediated by a reduction in available dietary calcium and decreased reproductive function. Feed intake decreases during HS (Mashaly et al., 2004; Azad et al., 2010; Quinteiro-Filho et al., 2010), which limits dietary calcium ingestion. Blood calcium concentration is further reduced by panting, a behavioral adaptation associated with overheating (Richards, 1970). Panting alters blood chemistry by decreasing the partial pressure of carbon dioxide and increasing pH value, which decreases the ionic calcium available for eggshell production (Bottje and Harrison, 1985; Odom et al., 1986; Arad et al., 1993; Usayran et al., 2001). High ambient temperature also impairs egg production by modifying a hen’s endocrine profile both indirectly via decreased feed intake...
and directly altering reproductive hormone synthesis and secretion. For example, high temperatures decrease 3β-hydroxysteroid dehydrogenase (Taira and Beck, 2006) and cytochrome P450 aromatase activity (Rozenboim et al., 2007), which respectively decrease progesterone (Novero et al., 1991; Elnagar et al., 2010) and 17-β estradiol concentrations (Rozenboim et al., 2007; Elnagar et al., 2010). Those hormones are required for normal reproductive activity and consequently regular egg laying (Oguntonji and Alabi, 2010).

Under elevated temperature conditions, birds change their behavior and physiological homeostasis to aid thermoregulation, thereby decreasing body temperature. In general, birds react similarly to HS but express individual variation in the intensity and duration of their responses. For example, body temperature and metabolic activity are regulated by the thyroid hormones, triiodothyronine (T3) and thyroxine (T4), and their balance. Previous studies report that T3 concentrations consistently decrease in hot environments (Yahav and Hurwitz, 1996; Star et al., 2008a; Elnagar et al., 2010), but the results of heat-mediated alterations on T4 concentrations are less consistent with the studies reporting decreases (Bobek et al., 1980), increases (Cogburn and Freeman, 1987; Sinurat et al., 1987; Elnagar et al., 2010), or no change (Mitchell and Carlisle, 1992). These heat-mediated changes in thyroid function further reduce reproductive activity (Péczely et al., 1980; Lien and Siopes, 1989).

Much of the individual variation in response to HS may have genetic bases, making it amenable to selective breeding of farm animals, including chickens. Most research on genetic differences in response to HS in chickens has concentrated on physical traits that allow birds to reduce heat load such as small body size and reduced feathering (Yunis and Cahaner, 1999; Deeb and Cahaner, 2001a,b; Sharifi et al., 2010a,b). However, research designed to investigate differential susceptibility of laying hens to HS based on genetic selection for physiological and behavioral traits is limited. Identification of genetically controlled physiological and behavioral traits that vary in response to HS will provide evidence that physiological and behavioral traits can augment the usage of physical traits to select for chickens that perform well under high ambient temperatures.

This study examined the HS responses of 2 strains of White Leghorn hens, Dekalb XL (DXL) and kind, gentle bird (KGB). Dekalb XL is a commercial strain individually selected for high egg production. The KGB strain, referred to as HGPS (high group production and survivability) in some previous studies (Cheng and Jefferson, 2008; Fahey and Cheng, 2008), was developed using group selection from an admixture of 6 White Leghorns strains, including the DXL strain (Craig and Muir, 1996a,b; Muir, 1996). The selection emphasized group productivity and survivability of related hens with intact beaks in colony cage housing. Previous research revealed KGB hens adapt better to a variety of stressors including transport (Cheng and Jefferson, 2008), social stress and disruption (Craig and Muir, 1996a,b; Fahey and Cheng, 2008), and short-term heat and cold stress (Hester et al., 1996a,b,c). Collectively, the selection applied on the chickens has created a unique strain, KGB, whose characteristic responses to various stressors differ from the parent strains, which include DXL. The current study was designed to examine the effects of genotypic variations on laying hens’ productivity and behavior following HS and the mechanisms underlying the differences.

**MATERIALS AND METHODS**

**Genetic Strains and Husbandry**

Ninety hens (48 DXL and 42 KGB hens) were randomly selected from a research flock reared for strain preservation. The hens were cage housed in pairs by strain at the Poultry Unit of Purdue University’s Animal Sciences Research and Education Center. At 28 wk of age, hens were removed from the flock and moved to the university’s Life Science Animal Building. There, they were randomly caged in pairs by strain in 2 adjacent, identical rooms. Twelve cages comprised a cage bank, and the 4 cage banks were balanced by strain. The cages were 76 cm × 52 cm × 48 cm (length × depth × height), which provided 1,976 cm² floor space per hen. The large floor space was used to ensure the observed differences between the strains resulted from HS rather than from a combination of multiple stressors, such as social stress from feather pecking and cannibalism (Craig and Muir, 1996a,b; Fahey and Cheng, 2008) and restriction stress from an overcrowded environment. In addition, hens were given 2 wk to adapt to the novel environment and to recover from stress experienced during transport. To ensure the rooms maintained similar temperature and humidity, the thermostats of both rooms were set at 23°C and the rooms were monitored daily. After the acclimation period, the rooms were randomly assigned to hot (H: mean = 32.6°C) or control (C: mean = 24.3°C) treatment with a similar humidity that ranged from 30 to 40% for 9 d. This resulted in 4 test groups: C-DXL, C-KGB, H-DXL, and H-KGB. Both rooms were artificially lit with a 16L:8D cycle. The hens had ad libitum access to food and water throughout the experiment.

All hens used in this experiment were housed and cared for under the approval of Purdue Animal Care and Use Committee (PACUC). All persons who handled or maintained hens were registered under PACUC (00-008–09).

**Egg Production and Quality**

Eggs were collected daily during the study. The per-cage hen-day egg production for the 9-d experimental period was calculated as (total number of eggs produced per cage/number of hens per cage) × 100. The percentage of egg breakage for the 9-d experimental
period was calculated as \((\text{total number of broken eggs per cage/total number of eggs produced per cage}) \times 100\). All the eggs collected on d 8 and 9 were weighed and their eggshell thickness measured with a coolant proof micrometer (Mitutoyo, Aurora, IL). Briefly, the eggshells were prepared by washing, overnight drying, and then having the membrane peeled off the interior surface. On each egg, measurements were taken from locations at the top, bottom, and middle of the eggshell. The mean of the 3 measurements was used as the eggshell thickness for the statistical analysis.

**Behavior**

Behavioral observations were conducted on d 1, 2, and 6 of the experiment. The behavior of both hens in each cage was digitally video-recorded and observed using 10-min scan sampling during two 2-h time blocks, one in the morning after the lights turned on and another in the evening before the lights turned off. Because it was not possible to distinguish between the hens within a cage, the behaviors of both hens were recorded according to a predefined ethogram (Table 1) and assigned to the cage. For each behavior, the data collected during the 3 d were averaged to obtain a mean for the treatment period. All behavior was recorded by individuals trained in observing and analyzing poultry behavior.

**Blood Sampling**

Prior to blood collection on d 8, both hens from randomly selected cages \((n = 10 \text{ DXL; 11 KGB cages})\) were removed and sedated with an intravenous jugular injection of 1 mL of sodium phenobarbital (30 mg/kg). Within 2 min of removal from cages, a 10-mL blood sample was collected from the hens by cardiac puncture before they were humanely euthanized by cervical dislocation. The blood samples were stored on ice until the plasma was separated by centrifugation at 700 \(\times g\) for 20 min at 4°C (Sorvall BC 3B Plus, Thermo Fisher Scientific, Waltham, MA). Following centrifugation, the supernatant plasma was collected and stored at \(-80^\circ\)C until analysis.

**Thyroid Hormones**

Total plasma \(T_3\) concentration was measured using commercial RIA kits (TKT31, Siemens, Washington, DC) per the manufacturer’s instructions. Samples were analyzed in duplicate. The assay had a calibration range of 20 to 600 ng/dL and an analytical sensitivity of 7 ng/dL. The intra- and interassay CV were 14.8 and 17.9%, respectively.

A commercial total \(T_4\) RIA kit (TKT41, Siemens, Washington, DC) was adapted for use with chickens by increasing the amount of plasma used from the manufacturer’s directions of 25 μL to 500 μL following validation. The assay was validated for linearity and parallelism using duplicated serial dilutions of 2 independent samples of pooled chicken plasma and a third sample composed of half of each independent sample. The remainder of the assay followed the manufacturer’s instructions. All samples were analyzed in duplicate. The assay had a calibration range of 1 to 24 μg/dL with an analytical sensitivity of 0.25 μg/dL. The sample concentrations derived from the standard curve were divided by 20 to adjust for the difference in amount of

<table>
<thead>
<tr>
<th>Behavior</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Posture</td>
<td>Both feet are in contact with the cage floor; no other body part is in contact with cage floor.</td>
</tr>
<tr>
<td>Sit</td>
<td>Most of the ventral region of the hen’s body is in contact with cage floor. No space is visible between the cage floor and the hen.</td>
</tr>
<tr>
<td>Action</td>
<td></td>
</tr>
<tr>
<td>Feed</td>
<td>Hen’s head is located inside feeder.</td>
</tr>
<tr>
<td>Drink</td>
<td>Hen’s beak is in contact with a nipple drinker.</td>
</tr>
<tr>
<td>Walk</td>
<td>Hen is in the process of taking multiple steps; this includes walking in place.</td>
</tr>
<tr>
<td>Rest</td>
<td>Hen is stationary.</td>
</tr>
<tr>
<td>Preen</td>
<td>Hen is gently pecking or scratching its own feathers.</td>
</tr>
<tr>
<td>Aggressive peck</td>
<td>Hen’s beak contacts another hen.</td>
</tr>
<tr>
<td>Wing position</td>
<td></td>
</tr>
<tr>
<td>Elevated</td>
<td>A space can be seen between hen’s wings and body.</td>
</tr>
<tr>
<td>Not elevated</td>
<td>There is no observable space between the hen’s wings and body.</td>
</tr>
<tr>
<td>Respiration</td>
<td></td>
</tr>
<tr>
<td>Panting</td>
<td>Hen’s beak is open and respiration rate is abnormally rapid.</td>
</tr>
<tr>
<td>Not panting</td>
<td>Hen’s beak is closed and respiration rate is normal.</td>
</tr>
</tbody>
</table>
Corticosterone

Plasma and yolk total corticosterone concentrations were measured using a commercially available RIA kit (#07–120–102 MP Biomedicals, Solon, OH). The kit’s minimum detectable dosage was 7.7 ng/mL. The assay’s cross-reactivity with progesterone was 0.02% and with 20α-dihydroprogesterone, pregnenolone, 17α-hydroxypregnenolone, and 17α-hydroxyprogesterone was <0.01%. Total plasma corticosterone concentrations were analyzed in duplicate following the protocol described by Cheng et al. (2001). The intra- and interassay CV were 17.7 and 17.0%, respectively.

Egg yolks were obtained from eggs collected on d 6 and frozen at −80°C until analysis. Yolk corticosterone was extracted using a protocol adapted from Robertson (2009). In brief, 1-g yolk was homogenized with 1 mL of deionized water and 1-g glass beads. A 2.5-mL aliquot of 100% methanol was added to a 500-μL aliquot of the homogenate, which was then vortexed and centrifuged. After centrifugation, a 1.5-mL aliquot of the sample was drawn off and 13.5 mL of deionized water was added to the aliquot. The solution was placed on a C-18 column, washed with deionized water, and the corticosterone extracted with methanol. The extracted sample was dried at 60°C using a Thermolyne dri-bath (Vernon Hills, IL). The pellet was resuspended in 330 μL of PBS with 0.25% BSA. Following the extraction, yolk corticosterone was measured using an adaptation of the protocol described by Cheng et al. (2001) by increasing the amount of sample from 20 to 60 μL. The assay was validated for linearity and parallelism using duplicated serial dilutions of 2 independent samples of pooled yolk extract and a third sample composed of half of each independent sample. Following validation, all samples were analyzed in duplicate. Concentrations of the samples were then divided by 3 to adjust for the difference in amount of plasma called for in the kit directions and the amount used. The intra- and interassay CV were 12.3 and 9.3%, respectively.

Statistical Analyses

The experimental design was an incomplete randomized block with temperature, strain, and their interactions as fixed effects and cage nested within temperature by strain as the random effect. Data were analyzed with a mixed model using the MIXED procedure in SAS 9.2 for Windows (SAS Institute Inc., Cary, NC). Data transformations were performed as necessary to normalize the distribution and equalize the variances of residuals and those $P$-values are presented. Drinking, walking, preening, aggressive pecking, T3, eggshell thickness, and egg breakage were square root transformed. Plasma corticosterone and egg weight were log10 transformed. Panting was angularly transformed. However, to aid interpretation, least squares means of the raw data ± SE are presented. The slice option was used to examine the effect of one independent variable within a level of the second independent variable. The Benjamini-Hochberg method was used to control the false discovery rate ($FDR$) due to multiple comparisons. The FDR was set at 0.05, and all $P$-values shown have been adjusted for multiple comparisons accordingly (Benjamini and Hochberg, 1995). Statistical significance was set at adjusted $(adj)$ $P \leq 0.05$ and with a trend at $0.05 < adj \ P \leq 0.10$.

RESULTS

Behavioral and Physical Measures Associated with Feeding

Overall, the H hens tended to spend less time feeding (Table 2) than C hens, and KGB hens spent more time feeding than DXL hens (F1, 43 = 10.00, adj $P = 0.02$). However, the crop fill weight (Table 2) did not differ by either temperature or strain and did not exhibit a temperature × strain interaction. Like crop fill weight,

<table>
<thead>
<tr>
<th>Test group</th>
<th>Feeding (%)</th>
<th>Crop fill (g)</th>
<th>BW (kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>C-DXL</td>
<td>32.50 ± 1.76</td>
<td>9.23 ± 2.37</td>
<td>1.48 ± 0.04</td>
</tr>
<tr>
<td>H-DXL</td>
<td>27.81 ± 1.78</td>
<td>1.81 ± 2.50</td>
<td>1.40 ± 0.04</td>
</tr>
<tr>
<td>C-KGB</td>
<td>38.12 ± 1.83</td>
<td>3.64 ± 2.26</td>
<td>1.43 ± 0.04</td>
</tr>
<tr>
<td>H-KGB</td>
<td>33.81 ± 1.95</td>
<td>6.76 ± 2.50</td>
<td>1.37 ± 0.04</td>
</tr>
</tbody>
</table>

For all data, least squares means ± SE of the raw data ($n = 10$ DXL, 11 KGB cages) are reported and $P$-values were adjusted to control the false discovery rate (Benjamini and Hochberg, 1995).

C-DXL = control-Dekalb XL (DXL); H-DXL = hot-Dekalb XL; C-KGB = control-kind, gentle bird (KGB); and H-KGB = hot-kind, gentle bird.
BW (Table 2) did not vary between temperatures or strains and also exhibited no temperature by strain interaction.

### Endocrine Measures

High temperature depressed the plasma concentrations of $T_3$ ($F_{1, 17} = 13.96$, adj $P = 0.01$; Table 3), especially among the DXL hens (adj $P = 0.01$). Compared with the C-KGB hens, the H-KGB hens had lower $T_3$ concentrations, but without statistical difference. There was neither a strain difference nor a temperature $\times$ strain interaction. Plasma $T_4$ concentration (Table 3) did not differ by temperature or strain, and there was no temperature $\times$ strain interaction. Neither plasma nor yolk corticosterone concentrations (Table 3) differed by temperature or strain, nor did they exhibit a temperature $\times$ strain interaction.

### Egg Production and Quality Measures

Neither relative ovarian weight nor number of mature follicles differed between temperatures or between strains, and neither showed a temperature $\times$ strain interaction (Table 4). Egg production (Table 4) was lower among H hens than C hens ($F_{1, 41} = 27.90$, adj $P = 0.001$). There was no strain difference in egg production and no temperature $\times$ strain interaction.

Egg weights (Table 5) were reduced by HS on both d 1 ($F_{1, 29} = 10.11$, adj $P = 0.02$) and d 9 ($F_{1, 32} = 25.79$, adj $P = 0.001$). However, there was neither a strain effect nor a temperature $\times$ strain interaction on either day. Eggs from H-DXL hens tended to weigh less than eggs from C-DXL hens on d 1 and weighed less on d 9 (adj $P = 0.007$). However, egg weight from H-KGB hens was reduced on d 9 only (adj $P = 0.007$).

Eggshell thickness (Table 5) was reduced on d 9 ($F_{1, 32} = 13.0$, adj $P = 0.007$), but not d 1 among

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### Table 3. The effects of heat stress on hens’ endocrinology

<table>
<thead>
<tr>
<th>Test group $^2$</th>
<th>$T_3$ $^{2,3}$ (ng/dL)</th>
<th>$T_4$ $^2$ (μg/dL)</th>
<th>Plasma cort $^{2,4}$ (ng/mL)</th>
<th>Yolk cort $^2$ (ng/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>C-DXL 160.43 ± 21.09$^a$</td>
<td>0.48 ± 0.17</td>
<td>7.06 ± 1.61</td>
<td>0.48 ± 0.03</td>
<td></td>
</tr>
<tr>
<td>H-DXL 61.83 ± 21.68$^b$</td>
<td>0.56 ± 0.17</td>
<td>5.63 ± 1.56</td>
<td>0.49 ± 0.04</td>
<td></td>
</tr>
<tr>
<td>C-KGB 144.98 ± 19.70</td>
<td>0.47 ± 0.16</td>
<td>7.19 ± 1.54</td>
<td>0.51 ± 0.04</td>
<td></td>
</tr>
<tr>
<td>H-KGB 96.75 ± 21.68</td>
<td>0.22 ± 0.17</td>
<td>7.42 ± 1.61</td>
<td>0.47 ± 0.04</td>
<td></td>
</tr>
</tbody>
</table>

Adjusted $P$-value

Temperature 0.012 0.755 0.755 0.750
Strain 0.755 0.565 0.755 0.948
Temperature $\times$ strain 0.371 0.580 0.755 0.647
C-DXL vs. H-DXL 0.012 0.836 0.684 0.945
C-KGB vs. H-KGB 0.303 0.565 0.979 0.582

$^a,b P < 0.05$. Least squares means within a strain and column with different superscripts differ from each other.

1For all data, least squares means ± SE of the raw data (n = 10 DXL, 11 KGB cages) are reported and $P$-values were adjusted to control the false discovery rate (Benjamini and Hochberg, 1995).

2C-DXL = control-Dekalb XL (DXL); H-DXL = hot-Dekalb XL; C-KGB = control-kind, gentle bird (KGB); and H-KGB = hot-kind, gentle bird; $T_3$ = triiodothyronine; $T_4$ = thyroxine; cort = corticosterone.

3Data were square root transformed for analysis.

4Data were log$_{10}$ transformed for analysis.

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### Table 4. The effects of heat stress on hens’ egg production measures

<table>
<thead>
<tr>
<th>Test group $^2$</th>
<th>Relative ovarian weight $^3$</th>
<th>Number mature follicles</th>
<th>Egg production $^3$ (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>C-DXL 30 ± 3</td>
<td>5.10 ± 0.63</td>
<td>80.95 ± 6.62$^A$</td>
<td></td>
</tr>
<tr>
<td>H-DXL 34 ± 3</td>
<td>6.10 ± 0.63</td>
<td>47.02 ± 6.62$^B$</td>
<td></td>
</tr>
<tr>
<td>C-KGB 27 ± 3</td>
<td>4.17 ± 0.57</td>
<td>79.22 ± 6.91$^A$</td>
<td></td>
</tr>
<tr>
<td>H-KGB 34 ± 3</td>
<td>5.33 ± 0.66</td>
<td>40.71 ± 7.25$^B$</td>
<td></td>
</tr>
</tbody>
</table>

Adjusted $P$-value

Temperature 0.239 0.299 0.001
Strain 0.755 0.424 0.755
Temperature $\times$ strain 0.759 0.948 0.838
C-DXL vs. H-DXL 0.573 0.552 0.007
C-KGB vs. H-KGB 0.299 0.303 0.004

$^A,B P < 0.01$. Least squares means within a strain and single column with different superscripts differ from each other.

1For all data, least squares means ± SE of the raw data (n = 10 DXL, 11 KGB cages) are reported and $P$-values were adjusted to control the false discovery rate (Benjamini and Hochberg, 1995).

2C-DXL = control-Dekalb XL (DXL); H-DXL = hot-Dekalb XL; C-KGB = control-kind, gentle bird (KGB); and H-KGB = hot-kind, gentle bird.

3Relative ovarian weight = absolute ovarian weight (g)/BW, kg; egg production = hen-day egg production for the 9-d experimental period was calculated as (total number of eggs produced per cage/number of hens per cage) $\times$ 100.
H hens when compared with C hens. The KGB hens were especially affected with eggshells from H-KGB hens (adj $P = 0.01$), but not H-DXL hens, being thinner than eggshells from their control hens. There was no strain effect or temperature × strain interaction on eggshell thickness at either d 1 or 9. Despite the reduction in eggshell thickness, temperature did not affect the percentages of broken eggs (Table 5). There was also no strain difference and no temperature × strain interaction in egg breakage.

**Behavioral Measures**

Hens in the H treatment spent more time with their wings elevated (Figure 1) than hens in the C treatment ($F_1, 43 = 234.99$, adj $P = 0.001$), but there was no difference between the strains and no strain × strain interaction. Panting was almost nonexistent among C-hens; therefore, only a comparison between H-DXL and H-KGB could be analyzed, which did not differ (Table 6). Overall, drinking (Figure 2) was observed more among H hens than C hens ($F_1, 43 = 12.47$, adj $P = 0.007$). The greatest difference in drinking behavior was between the H-KGB and C-KGB hens (adj $P = 0.04$), whereas the H-DXL drank more numerically, but not statistically, than their controls. There was no strain difference in drinking behavior and no temperature × strain interaction.

Under high temperatures, walking (Table 6) decreased ($F_1, 43 = 33.73$, adj $P = 0.001$), whereas resting (Table 6) increased ($F_1, 43 = 44.80$, adj $P = 0.001$) among H hens compared with C hens. There was no effect of strain and no temperature × strain interaction on the amount of time spent performing either behavior. Sitting and standing behaviors (Table 6) did not differ by temperature or strain, and there was no temperature × strain interaction. Neither preening behavior nor aggressive pecking differed by temperature or strain, and neither showed a temperature × strain interaction (Table 6).

**DISCUSSION**

The KGB hens spent more time feeding than the DXL hens; however, the greater time spent feeding did not result in differential crop fill weight or BW. Previous research studies with several broiler lines showed that phenotypic and genetic effects on feeding behav-

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**Table 5. The effects of heat stress on egg quality**

<table>
<thead>
<tr>
<th>Test group</th>
<th>Egg weight $^3$ (g)</th>
<th>Shell thickness $^4$ (μm)</th>
<th>Egg breakage $^5$ (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ED 6</td>
<td>ED 9</td>
<td>ED 1</td>
</tr>
<tr>
<td>C-DXL</td>
<td>51.30 ± 1.14 $^x$</td>
<td>49.83 ± 1.11 $^A$</td>
<td>338 ± 9</td>
</tr>
<tr>
<td>H-DXL</td>
<td>46.38 ± 1.52 $^y$</td>
<td>44.02 ± 1.15 $^B$</td>
<td>331 ± 13</td>
</tr>
<tr>
<td>C-KGB</td>
<td>48.54 ± 1.10</td>
<td>49.58 ± 1.11 $^A$</td>
<td>343 ± 9</td>
</tr>
<tr>
<td>H-KGB</td>
<td>44.83 ± 1.59</td>
<td>42.91 ± 1.41 $^B$</td>
<td>325 ± 13</td>
</tr>
</tbody>
</table>

Temperature: 0.021 0.001 0.552 0.007 0.116
Strain: 0.360 0.755 0.973 0.948 0.162
Temperature × strain: 0.759 0.847 0.759 0.362 0.755
C-DXL vs. H-DXL: 0.077 0.007 0.759 0.303 0.172
C-KGB vs. H-KGB: 0.246 0.007 0.552 0.012 0.470

$^A,B P < 0.01, ^a,b P < 0.05,$ and $^x,y 0.05 < P < 0.10.$ Superscripts indicate the probability of a statistical difference between least squares means within a strain and column.

1For all data, least squares means ± SE of the raw data (n = 10 DXL, 11 KGB cages) are reported and P-values were adjusted to control the false discovery rate (Benjamini and Hochberg, 1995).

$^2$C-DXL = control-Dekalb XL (DXL); H-DXL = hot-Dekalb XL; C-KGB = control-kind, gentle bird (KGB); and H-KGB = hot-kind, gentle bird.

$^3$Data were log$_{10}$ transformed for analysis.

$^4$Data were square root transformed for analysis.

$^5$Egg breakage for the 9-d experimental period was calculated as (total number of broken eggs per cage/total number of eggs produced per cage) × 100.

$^6$ED = experimental day.

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**Figure 1.** Wing elevation. Both H-DXL hens and H-KGB hens spent more time with their wings lifted than their respective controls [**adjusted (adj) $P = 0.001$]. There was no difference between strains (adj $P = 0.58$) and no temperature × strain interaction (adj $P = 0.95$). C-DXL = control-Dekalb XL; H-DXL = hot-Dekalb XL; C-KGB = control-kind, gentle bird; and H-KGB = hot-kind, gentle bird. The P-values were adjusted to control the false discovery rate (Benjamini and Hochberg, 1995).
iors were low and genetic correlations with various performance measures including BW were also low (Howie et al., 2009, 2011). Therefore, the relationship between the times spent feeding and other feed-related outcomes may not be very strong.

In this study, high ambient temperature tended to decrease the time spent feeding among hens from both strains, but did not affect crop fill weight or BW. These results were surprising because HS has previously been shown to decrease both feed intake and BW of various strains of laying hens (Franco-Jimenez et al., 2007; Star et al., 2008b). Differences in genetic backgrounds and the stressor used among the studies may contribute to the variation in results (Fox, 1951; Gowe et al., 2008).

In addition, the space allowance used in this study was 1,976 cm²/hen to minimize the effects of social and restriction stress. This space is approximately 2 to 3× greater than those used in the previous research studies and 4× greater than the minimum recommended stocking density (FASS, 2010). Under high ambient temperatures, core body temperature increases with increased stocking density (Green and Xin, 2009); therefore, the risk of BW decrease related to reduced feed intake may also increase, resulting from accumulated stressors such as heat, social, and restriction stress.

The thyroid system is among the systems most affected by HS (Simurat et al., 1987; Elnagar et al., 2010). Decreases in thyroid hormone activity impair reproductive functions of female turkeys and quail (Péczely et al., 1980; Lien and Siopes, 1989). Reduced feed intake decreases circulating concentrations of T₃ and increases T₄ concentrations (Klandorf and Harvey, 1985) and thus may often contribute to an indirect decrease in thyroid activity under high ambient temperature. However, in this study, HS appears to have directly affected thyroid activity. Compared with the C hens, H hens had reduced T₃ concentrations without changes to feed intake. Like these results, Elnagar et al. (2010) reported that the decreased T₃ concentrations were affected by HS directly and not mediated through reduced feed intake.

Unlike the reduction in T₃ concentration, T₄ concentration was not affected by high ambient temperature in this study. Previous studies have shown that HS-induced changes in T₄ concentrations are less consistent than changes in T₃ concentrations. High temperature has been reported to increase (Cogburn and Freeman, 1987; Elnagar et al., 2010), decrease (Williamson et al., 1985; Sohail et al., 2010), or not alter (Mitchell and Carlisle, 1992) the T₄ concentration of chickens. The various results may be associated with experimental differences among the ages and strains of the birds and

### Table 6. The effects of heat stress on hens’ respiration, posture, and locomotion

<table>
<thead>
<tr>
<th>Test group²</th>
<th>Panting³ (%)</th>
<th>Walking⁴ (%)</th>
<th>Resting (%)</th>
<th>Sitting (%)</th>
<th>Standing (%)</th>
<th>Preening⁴ (%)</th>
<th>Aggression⁴ (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>C-DXL</td>
<td>NA</td>
<td>18.40 ± 1.52A</td>
<td>27.28 ± 1.72B</td>
<td>16.23 ± 1.86</td>
<td>83.32 ± 1.91</td>
<td>5.17 ± 0.79</td>
<td>0.98 ± 0.31</td>
</tr>
<tr>
<td>H-DXL</td>
<td>77.44 ± 3.21</td>
<td>9.90 ± 1.55B</td>
<td>38.68 ± 1.74A</td>
<td>11.41 ± 1.93</td>
<td>88.10 ± 1.97</td>
<td>5.22 ± 0.80</td>
<td>0.32 ± 0.32</td>
</tr>
<tr>
<td>C-KGB</td>
<td>NA</td>
<td>20.84 ± 1.59B</td>
<td>23.98 ± 1.79B</td>
<td>11.14 ± 1.94</td>
<td>84.41 ± 1.99</td>
<td>4.21 ± 0.82</td>
<td>0.56 ± 0.32</td>
</tr>
<tr>
<td>H-KGB</td>
<td>86.18 ± 3.44</td>
<td>10.87 ± 1.70B</td>
<td>36.56 ± 1.91A</td>
<td>12.82 ± 2.11</td>
<td>86.80 ± 2.16</td>
<td>3.09 ± 0.87</td>
<td>0.40 ± 0.35</td>
</tr>
</tbody>
</table>

Adjusted P-value

Temperature NA | 0.001 | 0.001 | 0.654 | 0.662 | 0.723 | 0.565
Strain 0.358 | 0.708 | 0.338 | 0.587 | 0.587 | 0.248 | 0.750

Temperature × strain NA | 0.709 | 0.838 | 0.299 | 0.303 | 0.709 | 0.709

C-DXL vs. H-DXL NA | 0.005 | 0.001 | 0.248 | 0.279 | 0.979 | 0.447
C-KGB vs. H-KGB NA | 0.001 | 0.001 | 0.755 | 0.755 | 0.587 | 0.886

A,B P< 0.01. Superscripts indicate the probability of a statistical difference between least squares means within a strain and column.

1For all data, least squares means ± SE of the raw data (n = 10 DXL, 11 KGB cages) are reported and P-values were adjusted to control the false discovery rate (Benjamini and Hochberg, 1995).

2C-DXL = control-Dekalb XL (DXL); H-DXL = hot-Dekalb XL; C-KGB = control-kind, gentle bird (KGB); and H-KGB = hot-kind, gentle bird.

3Data were angularly transformed for analysis.

4Data were square root transformed for analysis.

5The C-hens were not observed panting; therefore, only data from the H-hens were analyzed.

![Figure 2. Drinking behavior. Overall, hens in the hot (H) treatment spent more time drinking than hens in the control (C) treatment [adjusted (adj) P = 0.007]. However, only the H-KGB hens (*adj P = 0.04) and not the H-DXL hens (adj P = 0.14) were different than their respective controls. C-DXL = control-Dekalb XL (DXL); H-DXL = hot-Dekalb XL; C-KGB = control-kind, gentle bird (KGB); and H-KGB = hot-kind, gentle bird. The P-values were adjusted to control the false discovery rate (Benjamini and Hochberg, 1995).](image)
the differences in the severity and duration of the heat treatments.

In this study, high ambient temperature did not affect either the yolk or plasma concentrations of corticosterone. Similarly, Hester et al. (1996b) reported these same hen strains did not show alterations in blood corticosterone concentrations under acute HS. In contrast, previous studies have shown increases in corticosterone concentration among both broilers (Soleimani et al., 2011) and layers (Star et al., 2008a) under short-term (Soleimani et al., 2011) or long-term HS (Quinteiro-Filho et al., 2010). The hypothalamic-pituitary axis is often activated under HS, but plasma concentrations of corticosterone may decline within a few hours of the initial temperature increase (Etches et al., 2008). Therefore, differences in corticosterone response to HS between this study and the previous ones may be related to genetic differences among the birds or the timing of sampling collection relative to temperature increase.

Egg production was reduced in both strains of hens under HS, which may be an adaptive stress response to conserve metabolic energy (Mumma et al., 2006). This hypothesis is further supported by the lack of differences in hen BW following HS in spite of reduced feeding time. In healthy animals, about 60% of the total energy intake is allocated to maintaining basal metabolic rate. In times of inadequate energy availability, which can occur with reduced feed intake, hens may shift energy usage away from biological functions not essential for survival, such as reproduction, by temporarily ceasing to lay eggs in order to maintain physiological integrity. The effects of high temperature on poultry egg production have been well established with examples from layers (Franco-Jimenez et al., 2007; Rozenboim et al., 2007; Star et al., 2008b), broiler breeders (Sharifi et al., 2010a,b), and Japanese quail (Ozcelik and Ozbey, 2004). These results are also consistent with previous research findings using the same hen strains under the various acute stressors of cold, heat, and handling (Hester et al., 1996c).

In addition to egg production, egg quality is often reduced under high ambient temperatures (Daghir, 2008). In this study, egg weight decreased in the H hens compared with the C hens with the DXL hens being affected sooner after temperature increase than the KGB hens. Similar to these results, decreased egg weight under high ambient temperatures has been reported extensively (de Andrade et al., 1977; Mashaly et al., 2004; Franco-Jimenez et al., 2007; Rozenboim et al., 2007; Star et al., 2008b), with some studies finding the magnitude of the response differed among strains (Star et al., 2008b). Like egg weight, overall eggshell thickness decreased in the H hens compared with the C hens; however, unlike egg production, eggs from the KGB hens were affected more than eggs from the DXL hens. High ambient temperature causing reduced eggshell thickness has been previously reported (Lin et al., 2004; Franco-Jimenez et al., 2007; Pereira et al., 2008). Similarly, differences between strains in egg quality have been found in hens under HS (Franco-Jimenez et al., 2007; Pereira et al., 2008; Melesse et al., 2010). These differences may be attributable to genetic variations altering physiological functions regulating egg formation (Buitenhuys et al., 2004; Zhang et al., 2005; Wright et al., 2006; Goraga et al., 2010).

Compared with the C hens, the H hens in this study reduced the percentages of time spent walking and increased the percentages of time spent resting. Reduced walking is a behavioral adaptation to HS to decrease body temperature (Gowe et al., 2008). Compared with the C hens, the H hens also spent a greater percentage of time with their wings elevated. Lifting wings away from the body exposes the lightly feathered apteria under the wings. This also reduces body temperature because the skin of the apteria contains near-surface blood vessels that promote heat transfer to the environment (Gerken et al., 2006).

Similarly, panting is among the behaviors exhibited by hens under HS because it increases evaporative cooling (Richards, 1970). In this study, panting was only observed in the hens under high ambient temperature. Panting increases water lost through evaporative cooling; consequently, chickens drink more under HS to replenish the lost water (Gowe et al., 2008). Like panting, drinking was greater in the H hens compared with the C hens. In addition to increasing drinking behavior, panting can also result in diminished egg quality as it increases the risk of respiratory alkalosis (Calder and Schmidt-Nielsen, 1968), which reduces calcium availability for eggshell production and results in thinner eggshells (Odom et al., 1986).

In conclusion, our results indicate that HS altered the hens’ behavior and negatively affected egg production and egg quality in both strains used in the study. However, the genetic background of the current strains affected the strength of many of the responses to HS. These results provide insights for future studies to determine if a selection program that incorporates the behavioral and physiological traits identified in this research would enhance the genetic selection for heat-resistant strains to improve hen health and production, especially under the current commercial conditions.

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