BREEDING AND GENETICS

Social Stress in Laying Hens: Differential Dopamine and Corticosterone Responses after Intermingling Different Genetic Strains of Chickens

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ABSTRACT White Leghorn chickens were genetically selected for high (HGPS) or low (LGPS) group productivity and survivability. The selection resulted in two genetic lines with marked opposite changes in cannibalism and flightiness when housed in multiple-colony battery cages without beak trimming. The objective of the study was to examine whether the genetic selection differentially affected the neuroendocrine system of chickens from different strains in response to social stress. Based on the previous studies, social stress was induced by randomly pairing 17-wk-old hens from three genetic lines, i.e., HGPS, LGPS, and Dekalb XL (DXL), to form three mixed-line combinations.

At 24 wk of age, the concentrations of plasma dopamine (DA) and corticosterone (CORT) showed no differences in DXL hens housed with HGPS or LGPS hens (P > 0.05). However, different regulations of DA and adrenal function were found between HGPS and LGPS hens when paired with DXL hens. Compared to HGPS hens, LGPS hens had greater levels of DA and CORT (P < 0.01 and P < 0.05, respectively). In addition, under the HGPS-LGPS social treatment, the concentrations of DA but not CORT were greater in LGPS hens than in HGPS hens (P < 0.05 and P > 0.05, respectively). The results indicated genetic selection for production and survivability differentially altered DA and CORT systems in response to social stress. The data suggested, compared to LGPS hens, HGPS hens had a better coping capability to social stress, which might have been responsible for their higher productivity and survivability.

(Key words: group selection, dopamine, corticosterone, chicken, well-being)

INTRODUCTION

Stress susceptibility of chickens is a major problem in the modern intensified poultry industry, and many managerial practices subject chickens to stress, such as chronic social stress under high-density artificial environments. Inability of chickens to adapt to their social environments results in a greater susceptibility to disease (Gross and Siegel, 1988; Awadalla, 1998) and an increase frequency of abnormal behavior, such as cannibalism, aggression, and feather pecking (Burger and Kaiser, 1996; Via, 1999; Bilcik and Keeling 2000; El-Lethey et al., 2000).

Social stress reaction is strain-specific interactions between dominance hierarchies and environmental effects (Haemisch and Gartner, 1994). When intermingling different species, strains, or different ages from the same strain of animals, including chickens, some strains become aggressive but others are peaceable (Savory, 1982; Noble et al., 1993; Mahagna et al., 1994; Gvaryahu et al., 1996; Harshfield and Grim, 1997). The different behavioral patterns may reflect the strain-specific environmental effects on social status and neuroendocrine states. Differential regulation of the dopaminergic system, i.e., dopamine (DA) concentrations and DA receptors, and adrenal function, i.e., concentrations of corticosterone (CORT) and hypertrophy of adrenal glands, has been found between dominant and subordinate animals (Blanchard et al., 1993; Shively, 1998; Fano et al., 2001). These results reveal an approach to combat stress and improve animal well-being through genetic selection, i.e., selection for genotypic or phenotypic feature associated with specific physiological and behavioral characteristics resulting in high resistance to stress (Siegel, 1989; Craig and Swanson, 1994; Newman, 1994; Siegel and Dunington, 1997; Muir and Craig, 1998; van der Waij et al., 2000).

Two genetically selected lines of White Leghorn chickens have been developed at Purdue University using a

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Abbreviation Key: CORT = corticosterone; DA = dopamine; DXL = Dekalb XL, a line of commercial laying hens; HGPS = hens with high group productivity and survivability; HPA = hypothalamic-pituitary-adrenal axis; LGPS = hens with low group productivity and survivability.
genetic selection program termed group selection that emphasized high (HGPS) or low (LGPS) group productivity and survivability of families housed together in colony cages without beak trimming (Craig and Muir, 1996a,b; Muir and Craig, 1998; Cheng et al., 2001a,b). Previous studies have shown that HGPS hens have less cannibalistic behavior and feather pecking with high resistance to heat and cold stimuli, compared to the hens from non-selected control strain from which the selected lines were developed and the hens from a commercial strain, Dekalb XL (DXL) (Hester et al., 1996a,b,c). Compared to the hens from the LGPS line, HGPS hens housed in single-hen cages have a greater cell-mediated immunity and lower baseline concentrations of DA (Cheng et al., 2001a,b).

Collectively, genetic selection has created two phenotypic chicken lines, each of which has unique features in physical indexes, behavioral patterns, and neuroendocrine expression. The selection-induced differential regulation of the neuroendocrine system and immunity could affect chicken capabilities to adapt to social stress, resulting from intensified housing environments.

Housing environment can be a source of social stress. Previous studies have shown that chronic social stress can be induced in chickens after 4 wk or less by mixing individuals from different strains (Savory, 1975; Noble et al., 1993; Gvaryahu et al., 1996). For the current study, chronic social stress was administered by pairing hens for 7 wk. Based on previous studies, the social dominant rank order among the hens from DXL and genetically selected lines was DXL > LGPS > HGPS (Craig and Muir, 1996a,b; Cheng et al., 2001a; Freire et al., 2001). Within the DXL-LGPS and DXL-HGPS pairs, a DXL hen was used as a standardized genetic competitor that has higher aggressive behavior (Craig et al., 1975) with greater mortality from cannibalism and flightiness in multiple-hen cages (Craig and Muir, 1996a,b).

The current study is one in a series investigating effects of genetic-environmental interactions on well-being in laying hens. The objective of the present experiment was to determine the effect of genetic selection on concentrations of DA and adrenal function in response to chronic social stress and to determine whether these parameters can be used as physiological indicators in the evaluation of animal well-being.

**MATERIALS AND METHODS**

**Genetic Lines**

The ninth generation of the genetically selected HGPS and LGPS lines and the DXL line were used as the genetic materials for the present study. The differences between the selected lines in productivity and survivability have been reported previously (Cheng et al., 2001a). In addition, the behavioral and physiological characteristics of the DXL have been studied (Craig et al., 1975; Craig and Muir, 1996a,b). Pullets of each genetic line were reared up to 17 wk of age under the same conditions at Purdue University’s Poultry farm.

**Chronic Social Stress**

Pullets with intact beak were reared under the same conditions using standard management practices in raised wire cages up to 17 wk of age. At 17 wk of age, hens from each line were randomly assigned to two-hen cages to form three mixed-line combinations (15 replicates). The cages provided 419 cm² per hen, which is comparable to the standard of United States commercial facilities (Federation of Animal Science Societies, 1999). Feed and water were provided ad libitum. Overhead lights were on daily from 0700 until 1900 h initially and were increased by 15 min/wk. Light duration was 13 h daily when the study was performed, when the hens reached 24 wk of age.

Chicken care guidelines were in strict accordance with the rules and regulations set by Federation of Animal Science Societies (1999). Experimental protocols were approved by the institutional Animal Care and Use Committee at Purdue University. Efforts were made to minimize animal suffering and the number of animals used.

**Body Weight and Adrenal Gland Weight**

Body weight was taken immediately after removing the hens from their cages. After blood samples were collected, birds were killed by cervical dislocation. Based on the asymmetric development of the adrenal glands between the right and left sides, and the irregular shape of the left adrenal gland resulting from development of the reproductive system, the right-side adrenal gland was dissected without fat and then immersed in 10% neutral buffered formalin. After fixation, the glands were trimmed to remove other extraneous material if there was a need. After excess buffer was removed with paper towels, the adrenal glands were weighed and represented as absolute and relative adrenal gland weights. The relative adrenal gland weight represented a ratio of ADRENAL GLAND WEIGHT to BW (mg/kg).

**Blood Sampling**

A 5-mL blood sample was collected from the brachial vein of each pullet into an EDTA-coated tube within 2 min of the bird being removed from its cage. Samples were centrifuged at 700 × g for 15 min at 20 C. Plasma was kept on ice for further processing or kept at −80 C until measurement.

**HPLC Assay**

A plasma catecholamine analysis kit was used to measure blood concentrations of DA. Duplicate plasma samples were acidified and deproteinized with 4 M perchloric acid. After centrifugation, the acid supernatants and inter-
nal standard dihydroxybenzylamine were added and absorbed onto an alumina minicolumn to bind the DA. The columns were then rinsed and eluted with the solutions supplied by the company. After injection of eluents into the reverse-phase columns, catechols were detected by liquid chromatography with a Coulombek II electrochemical detection. The mobile phase (75 mM Na₂HPO₄, 1.7 mM 1-octanesulphonic acid, 25 μM EDTA, 10% CH₃CN, and 100 μL/L triethylamine, adjusted to pH 3.00 with phosphoric acid) flow rate was 1.3 mL/min. The concentrations of DA were calculated from a reference curve made using supplied standards and were presented as picograms per milliliter. The dopamine index was presented as the percentage of the mean DA concentrations of the DXL hens divided by the mean DA concentrations of the DXL hens. The dopamine index was calculated as the percentage of the mean DA concentrations of HGPS or LGPS hens divided by the mean DA concentrations of the DXL hens. The CORT index was calculated as the percentage of the mean CORT concentrations of the DXL hens.

### Radioimmunoassay

Total plasma CORT was measured in duplicate using a commercial 125I CORT radioimmunoassay kit with a modification based on the company’s suggestion for use with chicken samples. In order to validate for parallelism and recovery for chickens, an adjustment of dilutions at 1 to 5, were made, i.e., 20 μL sample to 80 μL steroid diluent. The concentrations of DA were calculated from a reference curve made using supplied standards and were presented as picograms per milliliter. The dopamine index was presented as the percentage of the mean DA concentrations of HGPS or LGPS hens divided by the mean DA concentrations of the DXL hens.

### Statistical Analyses

The data were analyzed using the general linear models procedure of SAS software (1992), based on a completely randomized design. Main effects included genetic lines, stress treatments, and interaction between main effects. Cages within each line were used as the experimental unit and hens within the cages were partitioned as a nested effect. Cage effects were conservatively tested at the 25% level of significance using within cage variation and were not significant. The two sources of variation were pooled before testing for line, stress, and interaction effects.

### RESULTS AND DISCUSSION

A genetic basis for different regulations of plasma DA concentrations and adrenal functions in response to social stress were found among the present genetic strains (Tables 1 and 2, $P < 0.01$ and $P < 0.05$, respectively). The results were consistent with data previously reported that domestication of animals is associated with hereditary reorganization of the neuroendocrine system (Naumenko et al., 1987; de Kloet et al., 1996; Ferris, 2000) and changes of neurochemical homeostasis (Bilzard et al., 1983; Balsa et al., 1996; Crusio, 1996; Davidson et al., 2000; Oquendo and Mann, 2000). The differential changes in the neuroendocrine functions among the present strains could be associated with the line’s unique productivity and survivability in a crowded social environment and specific resistance to stimuli reported previously (Craig et al., 1975; Craig and Muir; 1996a,b; Hester et al., 1996c; Cheng et al., 2001a).

Previous studies have provided evidence that the neuroendocrine system is regulated differently by stimuli in the animals, which is dependent on the interactions between genes and environments. Social stress, like a variety of stressful stimuli, acts through the central ner-
vouss system to trigger release of neurotransmitters and stress hormones, such as DA and CORT, from the sympa-thoadrenal and hypothalamic-pituitary-adrenal (HPA) axes. Disregulation of DA and CORT, including their concentrations and metabolites as well as densities of their receptors, has been associated with abnormal behavior and various productivities in mammals (Sharp et al., 1984; Tuomisto and Mannisto, 1985; Lewis et al., 1994; Berman and Coccaro, 1998; Depue and Collins, 1999). There is evidence that the function of the avian neuroen-doctrine system in response to stimuli is analogous to that in mammals (Harvey et al., 1984; Lowndes and Stewart, 1994; Muir, 1999; Tramontin and Brenowitz, 2000). In birds, as in rodents, stress-induced behavioral sensitivity is based on changes in the neuroendocrine system (Mills and Faure, 1991; Jones et al., 1992, 1994; Jones and Sat-terlee, 1996), which in turn affects bird coping styles and well-being (Lamont, 1994; Siegel, 1995; Mench and Dun-can 1998). Understanding effects of interaction between environment and genes on the neuroendocrine homeostasis in chickens is critical in preventing harmful behaviors and enhancing productivity associated with welfare in poultry husbandry (Mench, 1992; Craig and Swanson, 1994; Muir and Craig, 1998).

Similar to the findings in the mammals, the present study revealed that there is species-specific regulation of the neuroendocrine system among the chicken strains. The differences in the neuroendocrine function could be related to the differences in behavioral patterns and productivi-ty and survivability among the present lines housed in the colony cages without beak trimming (Craig et al., 1975; Craig and Muir, 1996a,b). The hypothesis is consistent with the previous findings that the HPA and sympathoadrenal axes control an animal’s behavioral patterns, production, and stress responses (Sotowska-Bro-chocka et al., 1994; Haller et al., 1997; Savory and Mann, 1997; Driscoll et al., 1998; Kuikka et al., 1998; Haller et al., 2000).

There were no differences in plasma DA and CORT concentrations as well as absolute and relative adrenal weights among DXL hens paired with the hens from HGPS or LGPS lines (Table 3; P > 0.05). The data further support the concept that the DXL line could be a reliable standardized genetic competitor in the present study. The relatively constant physiological features of DXL hens could be related to their higher social rank, compared to the hens from either HGPS or LGPS line in the present social environment. Similar to the present results, different regulations of the neuroendocrine system in response to social stress, based on its position in the structure of the group, have been reported in rodents (Kollack-Walker et al., 1997), pigs (Tuchscherer et al., 1998), and monkeys (Eberhart et al., 1985; Yodyingyuda, et al., 1985). All of the latter studies have shown that the physiological homeostasis, including cortisol, is not affected significantly in the dominant animals compared to the subordinate ones.

The LGPS hens, compared to HGPS hens, had greater concentrations of both DA and CORT when paired with DXL hens (Table 1, P < 0.01 and P < 0.05, respectively). These results could indicate that, when social partnering with a dominant competitor, the hens from the LGPS line were more stressed than those from the HGPS line. A parallel study has shown that, under the same treatment, aggressive pecks (i.e., peck on the head) and damaging pecks (i.e., peck on the other regions of body) were greater from the hens of the LGPS line than those of the HGPS line (Freire et al., 2001). Enhanced feather pecking and aggressive and cannibalistic behaviors could result from stress in chickens (Via, 1999; El-Lethey et al., 2000). In agreement with the present findings, genetically related differential social stress responses have been found when intermingling chickens from different social groups or genetic strains, i.e., some strains showed social stress reac-tions, others were peaceful (Savory, 1982; Mahagna et al., 1994; Gvaryahu et al., 1996). In rodents, when social contacts between the rats from different stains, such as Fischer 344, Sprague-Dawley, and Lewis, only the rats from the Fisher 344 showed stress-induced enhanced activation of the HPA axis, i.e., increase of ACTH and CORT concentrations (Dhabhar et al., 1997; Herman et al., 1999). In humans, enhanced output of cortisol was higher in groups that are in an intermediate cultural status than in those who follow a traditional way of life (Editorial, 1994).

Adrenal gland weights, but not BW, were affected differently among the chickens from the three genetic lines in response to the social stress (Table 2). The HGPS and LGPS hens, compared to hens from DXL line, had heavier

<table>
<thead>
<tr>
<th>Group</th>
<th>AGW (mg)</th>
<th>BW (kg)</th>
<th>AGW/BW (mg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>DXL</td>
<td>66±3</td>
<td>1.659±0.051</td>
<td>3.98±0.38</td>
</tr>
<tr>
<td>HGPS</td>
<td>75±4</td>
<td>1.526±0.091</td>
<td>4.91±0.26</td>
</tr>
<tr>
<td>LGPS</td>
<td>74±4</td>
<td>1.537±0.047</td>
<td>4.81±0.26</td>
</tr>
<tr>
<td>LGPS:DXL (%)</td>
<td>108</td>
<td>99</td>
<td>102</td>
</tr>
<tr>
<td>HGPS:DXL (%)</td>
<td>114</td>
<td>92</td>
<td>123</td>
</tr>
<tr>
<td>LGPS:DXL (%)</td>
<td>112</td>
<td>93</td>
<td>120</td>
</tr>
</tbody>
</table>

*Means within a column with no common superscript are statistically different (P < 0.05).

DXL = commercial Dekalb XL line; HGPS and LGPS = lines that were selected for high or low productivity and survivability, respectively.

AGW = adrenal gland weight; AGW/BW = relative adrenal gland weight.
adrenal glands in absolute and relative weights (Table 2; \( P < 0.05 \)), which suggests there is a hierarchical structure of the HPA axis in a line. Similar to the current findings, Hester et al. (1996a) reported that HGPS hens (named selected line in their study) had hypertrophic adrenal glands compared to DXL hens. However, there were no differences in absolute or relative adrenal weights between hens from HGPS and LGPS lines when paired with DXL hens (Table 2; \( P > 0.05 \)). The data are opposite to previous findings that HGPS hens have heavier adrenal glands than LGPS hens housed in single-hen cages (Cheng et al., 2001a). The different results between HGPS and LGPS hens housed in two-hen vs. single-hen cages could be related to stress-induced hypertrophy of adrenal glands in LGPS hens. The stress-induced shift of the adrenal glands in LGPS hens results in the disappearance of the differences between the present selected lines. These data further suggested that social encounters with aggressive competitors were physiologically more stressful in hens from LGPS line than those from HGPS line. Similar to the current results, Gross and Siegel (1985) reported there are genetically based differential stress reactions in the chicken lines selected for high or low plasma CORT response to social stress. The chickens from the high CORT response line, compared to those from the low CORT response line, were more stressed when mixed with strangers, as evidenced by lower feed efficiencies and less effective immunity (Gross and Colman, 1971; Gross and Siegel, 1985).

Genetically related differential regulation of the neuroendocrine system between HGPS and LGPS hens was also evidenced when the HGPS and LGPS hens were paired with each other (Table 4). Although the DA concentrations were less in HGPS and LGPS hens compared to the concentrations in HGPS-DXL and LGPS-DXL social treatments, respectively (Tables 1 and 4), LGPS hens had greater levels of DA than those of HGPS hens (Table 4; \( P < 0.05 \)). In contrast, there were no differences in CORT concentrations or adrenal gland weights between HGPS and LGPS hens. These results could indicate that chickens mixed with each other and initially selected from the same genetic line are less stressed than when paired with an aggressive competitor from a different genetic stain. However, the data suggested that under the lower-stress environment, compared to HGPS hens, LGPS hens were still in a stressed status with greater concentrations of DA.

The adrenal function was not different between HGPS and LGPS hens in the HGPS-LGPS treatment, which could suggest stress-induced shift of the adrenal response of LGPS hens from the levels in the single-hen treatment reported previously (68 vs. 60 mg, paired with HGPS vs. single-hen treatment; Cheng et al., 2001a). Similar to the current finding, Tolman (1968) and Meunier-Salaun and Faure (1984) reported that the chickens housed together from the same line had less stress than hens intermingled from different genetic lines. The latter social environment caused more severe social stress (Savory, 1982; Noble et al., 1993; Mahagna et al., 1994; Gvaryahu et al., 1996).

Bartolomucci et al. (2001) found that mice reared in a group of siblings since weaning were less stressed compared to mice housed with strangers when they become adults, and there was no difference in the CORT levels between dominants and subordinates from the sibling group in response to the open-field test. When social contacts between rats from different stains, such as from Fischer 344, Sprague-Dawley, and Lewis, were made, stress-induced enhanced activation of the HPA axis was evidenced by greater ACTH and CORT concentrations in the Fischer 344 strain only (Dhabhar et al., 1997; Herman et al., 1999). The strain differences in response to social stress could be related to genetically based differential regulation of the HPA axis and its capability of adaptation. Different inheritable cannibalistic and aggressive actions and resistance to stress have been found in various

### TABLE 4. Genetically based changes of dopamine (DA) and corticosterone (CORT) concentrations (\( \bar{x} \pm SD \)) in selected laying hens

<table>
<thead>
<tr>
<th>Group</th>
<th>CORT (ng/mL)</th>
<th>AGW(^2) (mg)</th>
<th>DA (pg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>HGPS(^i)</td>
<td>8.9 ± 2.2</td>
<td>67 ± 4</td>
<td>40 ± 10</td>
</tr>
<tr>
<td>LGPS</td>
<td>10.9 ± 2.9</td>
<td>68 ± 4</td>
<td>80(^b) ± 20</td>
</tr>
</tbody>
</table>

\(^{i}\) HGPS and LGPS paired with each other. HGPS and LGPS = lines that were selected from high and low productivity and survivability, respectively.

\(^{b}\) AGW = adrenal gland weight.

\(^{\bar{x}}\) Means within a column with no common significantly different (\( P < 0.05 \)).
stains of chickens (Hughes and Duncan, 1972; Higgins and Calnek, 1975; Carsia and Weber, 1986; Muir and Craig, 1988; Kjaer and Sorensen, 1997; Savory and Mann, 1997). These differences could reflect individual heritable coping strategies (Benus et al., 1991).

It remains unclear as to the mechanisms underlying the different regulation of plasma DA and adrenal function among the chicken lines. Other studies of various species show that alterations of DA could be related to various situations, such as clinical diseases including pituitary tumors (Arafah and Nasrallah, 2001; Velkeniers, 2001), heart failure in humans (Potluri et al., 2001), and rewarded learning in humans and animals (Grace, 2000; Wise, 2000).

Recent studies have indicated chronic stress-triggered activation of genes that encode catecholamines-synthesizing enzymes in the central nervous system and peripheral catecholaminergic systems, including the adrenal medulla (Nankova and Sabban, 1999; Sabban and Kvetnak, 2001). Similar to the present results, studies in humans and rodents have shown that plasma DA levels closely reflect the activity in the sympathoadrenal and HPA axes (Lackovic and Relja, 1983; Smit et al., 1995). Peripheral DA levels have been used to assess the intensity in the sympathetic response to stimuli (Muir et al., 1995; Pani et al., 2000). In addition, DA plays an inherent role in the control of the endocrine system homeostasis (Muir et al., 1989) and behavior, such as fear and social anxiety (Matthew et al., 2001).

In conclusion, the present study demonstrated that genetic selection for high and low group productivity and survivability affects regulation of the dopaminergic and adrenal functions in response to social stress. The line differences in blood concentrations of DA and adrenal function could be associated with unique characteristic of behavioral patterns, productive capability, and coping ability to social stimulation. The unique homeostatic characteristics of each selected line may provide a neurobiological basis for investigating effects of interactions of genetic factors and environments on animal well-being. The results suggested that evaluation of animal well-being should rely on multiple indicators rather than a single biological response, and the changes of dopaminergic system could be used as an indicator of stress in farm animals.

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