

# Social Stress in Laying Hens: Differential Effect of Stress on Plasma Dopamine Concentrations and Adrenal Function in Genetically Selected Chickens

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**ABSTRACT** Genetic selection for high or low group productivity and survivability (HGPS, LGPS) has created two phenotypically distinct chicken lines. Each line has unique characteristics in behavioral and physiological adaptability to multiple-bird cage system. The present study was designed to examine whether these differences reflect genetic variation in the control of plasma dopamine (DA) concentrations and adrenal function in response to social stress. Chickens from the HGPS and LGPS lines were randomly assigned to single- or 10-bird cages at 17 wk of age. The 10-bird cages were the same as those used in the development of the two lines. Differences in regulation of DA concentrations and adrenal function in response to different social environments were measured between the two lines when the study was conducted at 24 wk of age. In the 10-bird cages, the HGPS line had

lower levels of DA ( $P < 0.05$ ) and heavier adrenal glands (AG,  $P < 0.05$ ) than those of the LGPS line, but concentrations of corticosterone (CORT) from the two lines were not significantly different. In the single-bird cages, DA levels in both lines were greater than in that of their siblings in the 10-bird cages, but a greater increase was found in the LGPS line ( $P < 0.01$  and  $P < 0.05$ , 405% vs. 293%). Likewise, both lines had lower concentrations of CORT ( $P < 0.05$ ) in the single- vs. 10-bird cages, but the AG were less heavy in the LGPS line but not in HGPS line in the single-bird cages ( $P < 0.05$ ). The results indicated that the two strains reacted differently in terms of their stress hormone levels in the two different environments. These differences could contribute to the behavioral and physiological differences existing between the two lines.

(Key words: genetic selection, social stress, dopamine, corticosterone, chicken)

2003 Poultry Science 82:192–198

## INTRODUCTION

Enhancement of productivity and survivability of farm animals is a major goal of husbandry management strategies. One approach to reach this goal is to reduce stress and increase adequate adaptation to stressors in livestock, including laying hens. Previous studies have shown that acute and chronic stress, such as social stress under different housing conditions, results in chickens with increased susceptibility to harmful pathogens (Awadalla, 1998) and in chickens that display abnormal behaviors, such as cannibalism, feather pecking, and aggression (Hughes and Wood-Gush, 1977; Via, 1999; Bilcik and Keeling, 2000; El-Lethey et al., 2000). These abnormal behaviors can lead to decreased productivity, physical and emotional suffering, and death.

Stress reaction in animals is controlled by the neuroendocrine systems. Especially, the hypothalamic-pituitary-adrenal (HPA) and sympathetic-medullary-adrenal (SMA) axes are thought to be the final common pathways in controlling an animal's ability to cope with its environment. These systems control animal well-being in response to stressors (Minton, 1994; Smit et al., 1995; Leng and Russell 1998; Demitrack and Crofford, 1998; Bugajski, 1999; Mannelli et al., 1999; Negrao et al., 2000; Ehlert et al., 2001). Stress responses of the HPA and SMA axes are mainly located at the adrenocortical and medulliadrenal levels, respectively (Van Loon and Sole, 1980; Takahashi and Kalin, 1991; Gomez et al., 1996; Bakshi and Kalin, 2000; Ehlert et al., 2001). Corticosterone (CORT), one of the hormones released from the adrenal cortex of the HPA axis, and plasma dopamine (DA), one of the catecholamines released from the peripheral systems including the medulliadrenal of the SMA axis, have multifunctional roles in

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Received for publication April 11, 2002.

Accepted for publication August 8, 2002.

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**Abbreviation Key:** AG = adrenal glands; CORT = corticosterone; DA = dopamine; HGPS = high group productivity and survivability; HPA = hypothalamic-pituitary-adrenal; LGPS = low group productivity and survivability; SMA = sympathetic-medullary-adrenal.

both normal and abnormal states (Hennessy, 1997). Both DA and CORT are functionally involved in the control of an organism's behavioral style, metabolic, endocrine, and immune function to ensure adequate coping strategies and well-being (Gaillard and Al-Damluji, 1987; Tempel and Leibowitz, 1994; Siegel et al., 1999; Haller et al., 2000; Pani et al., 2000). In response to environmental exposure, genetic variation in stress reactions of animals, such as pigs and rodents, have been identified to be of adrenal origin (Van Loon and Sole, 1980; Hessing et al., 1994; Dhabhar et al., 1997; Desautels et al., 1999).

In poultry, stress-induced behavioral and physiological changes could be similar to those found in mammals, since there is evidence that stress-induced alterations of the neuroendocrine system are analogous between birds and mammals (Harvey et al., 1984). Similar to studies in mammals (Gentsch et al., 1981; Hessing et al., 1994; Hennessy, 1997; Breivik et al., 2000), several hormones including CORT have been used as a marker of stress response in genetic selection of birds (Brown and Nestor, 1974; Gross and Siegel, 1985; Jones et al., 1994; Sufka and Weed, 1994; Satterlee and Jones, 1997; Dmitriev et al., 2001). Hereditarily differential adrenal function in response to stress has been found in birds, which correlates birds' physical features, such as growth rate, egg production, and semen yield (Bayyari et al., 1997; Nestor et al., 2000; Dmitriev et al., 2001). The results of these studies are consistent with the hypothesis that the genotype or phenotype of an animal influences its hormonal reaction to stimulations, which in turn alters the animal's behavioral adaptability and well-being (Siegel, 1995; Mench and Duncan, 1998). Understanding a genetic basis for animals' different responses to stress is critical in preventing harmful managerial practices and enhancing productivity in the poultry industry (Mench, 1992; Craig and Swanson, 1994).

A genetic basis for different regulation of behavioral styles and physical parameters in response to various stimuli has been found in chickens selected for high or low group productivity and survivability (HGPS, LPS) when housed in colony cages (Craig and Muir, 1996a,b; Muir and Craig, 1998; Cheng et al., 2001b). Compared to the LPS line and a commercial strain, Dekalb XL, the HGPS line has been shown to have improved rate of lay, survival, and feather score as well as reduced cannibalism and flightiness (Craig and Muir, 1996a,b; Cheng et al., 2001b). The HGPS line also has better and faster adaptation to various stressors such as social, handling, cold, and heat in multiple-bird cages (Hester et al., 1996b,c). In addition, the HGPS hens display a greater cell-mediated immunity with a higher ratio of CD4:CD8 cells, whereas the LPS hens exhibit eosinophilia and heterophilia and have a greater heterophil:lymphocyte ratio in the single-bird cage (Cheng et al., 2001b). Overall, selection has created the two lines to have significantly different phenotypes, including unique physical parameters, behavioral styles, and resistance to stressors. These differences could be due to selection resulting in alterations of the HPA axis, SMA axis, or both. However, this hypothesis has not been examined. The objectives of the present experiment were to examine the

effect of genetic selection on regulation of DA and adrenal systems in response to different social environments and to investigate the hypotheses that chickens' coping capability and productivity are based on the interaction of social structure and genotypic features.

## MATERIALS AND METHODS

### *Genetic Lines*

The ninth generation of the HGPS and LPS lines developed at Purdue University was used as the genetic material for the current study. The differences in productivity and survivability of the two lines have been reported previously (Cheng et al., 2001a).

### *Social Treatment*

Layer pullets without beak trimming 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, pullets from each line were randomly assigned to single- or 10-bird cages (15 replicates). The cages provided 1,084 and 542 cm<sup>2</sup> per pullet with dimensions of 30.5 × 35.6 × 45.7 cm and 152.5 × 178 × 228.5 cm (W × L × H), respectively. Each cage provided feeder space at 30.5 and 15.5 cm per pullet, respectively, which allowed all pullets to eat at the same time in the 10-bird cages. Each 10-bird cage provides a ratio of one nipple drinker to two pullets. Feed and water were provided for ad libitum consumption. Overhead lights were on daily from 0700 until 1900 h initially and were increased by 15 min/wk until a light duration of 13 h daily was reached when the pullets were 24 wk of age.

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

### *Body Weight and Adrenal Gland Weight*

The study was conducted when pullets became 24 wk of age. Body weights were taken immediately after removing the pullets from their cages. Based on the asymmetric development of the adrenal glands (AG) between the right and left side and the irregular shape of the left AG resulting from development of the reproductive system, the right side AG was dissected without fat and then immersed in 10% neutral buffered formalin. Following fixation, excess buffer was removed with paper towels, then weights of the AG were measured and represented as absolute and relative adrenal gland weight. The relative adrenal gland weight represents a ratio of absolute adrenal gland weight to BW (mg/g).

### *Blood Sampling*

A 5 mL blood sample was collected into an ethylenediaminetetraacetic acid-coated tube from the brachial vein

of each hen within 2 min of removal from its cage. Samples were centrifuged at  $700 \times g$  for 15 min at 20 C. Plasma was kept on ice for further processing or kept at  $-80$  C until measurement.

### HPLC Assay

The ESA plasma catecholamine analysis kit<sup>2</sup> 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 internal 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.<sup>3</sup> Following injection of eluents into the reverse-phase columns, catechols were detected by liquid chromatography with electrochemical detection. The mobile phase (75 mM  $\text{Na}_2\text{HPO}_4$ , 1.7 mM 1-octanesulfonic acid, 25  $\mu\text{M}$  EDTA, 10%  $\text{CH}_3\text{CN}$ , and 100  $\mu\text{L/L}$  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 percentage of the mean DA concentrations of HGPS or LGPS hens divided by the mean DA supplied standards and were presented as nanograms per milliliter.

### Radioimmunoassay

Total plasma CORT was measured in duplicate using a commercial <sup>125</sup>I CORT radioimmunoassay kit<sup>3</sup> with a modification based on the company's suggestion for use in chicken samples. In order to validate for parallelism and recovery of chickens, an adjustment of dilutions at 1 to 5 were made, i.e., 20  $\mu\text{L}$  sample to 80  $\mu\text{L}$  steroid diluent. The concentration of CORT was calculated from a reference curve that ranged from 0.1 ng/mL (95.6% binding) to 4.0 ng/mL (15.1% binding) and the correlation coefficient was 0.9995. Recovery of exogenous CORT was measured by adding known amounts of unlabeled CORT to aliquots of steroid diluent to produce theoretical concentrations of 0.5, 1.0 and 2.0 ng/mL and to result in recovered concentration at 0.48, 1.08, and 1.97 ng/mL, respectively. The sensitivity of the assay was 0.02 ng/mL. Within and between-assay coefficients of variation were 7.6 and 8.7%, respectively.

### Statistical Analysis

The data were analyzed using the GLM procedure of SAS (SAS Institute, 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 signifi-

cance using within cage variation and found nonsignificant. The two sources of variation were pooled before testing for line, stress, and interaction effects.

## RESULTS AND DISCUSSION

### *Effect of Genetic-Social Environmental Interactions on Adrenal Function*

The present study demonstrated that social stress affected adrenal function differently between the reversely selected HGPS and LGPS lines. Although concentrations of CORT were not different between the two lines under the same treatment, both lines had less CORT levels in the single-bird cages than in the 10-bird cages (Table 1,  $P < 0.05$ ). This result is consistent with the findings that hens raised individually may be under relatively low social stress (Gross and Siegel, 1980). Up-regulation of CORT has been used as an indicator of stress response in animals including chickens (Gross and Siegel, 1985; Koelkebeck et al., 1986; McCormick et al., 1998; Stohr et al., 1999; Keeney et al., 2001). In laying hens, a positive correlation exists between population density and CORT concentrations (Mashaly et al., 1984). Stocking density has been identified to be a social stressor in hens (Blokhuis and Wiepkema, 1998; Savory et al., 1999). Lower resting CORT levels have also been found between rats housed in single vs. group cages (Barrett and Stockham, 1965; File and Peet, 1980).

Body weight did not differ between the lines in the same treatment or between treatments (Table 2,  $P > 0.05$ ), but both absolute and relative weight of the AG were heavier in the pullets from the HGPS line than those from the LGPS line in both single- and 10-bird cages ( $P < 0.05$ ). In addition, weight of the AG was not different in the HGPS pullets house in single- vs. 10-bird cages (Table 2), while the LGPS pullets had heavier AG in the 10-bird cages than in the single-bird cages ( $P < 0.05$ ). The difference in the adrenal response between the selected lines in response to different housing environments suggests that pullets from the HGPS line but not from the LGPS line have developed a capacity of acclimation of stress-induced adrenal reaction, since the strains were originally selected from the colony caged under continuous exposure to social stress. In contrast, the heavier adrenal glands in the LGPS pullets in the 10- vs. single-bird cages could indicate that the 10-bird environment (a crowded and socially competitive environment) is physiologically more stressful in the LGPS pullets than in the HGPS pullets.

The hereditary adrenal hypertrophy in HGPS pullets could be associated with their high productivity and survivability. Similar to current results, Hester et al. (1996b) reported that the HGPS hens (termed selected line) had heavier AG and better coping abilities to various stressors than the hens from both the control line and the commercial Dekalb XL line housed under either single- or multiple-bird cages. In supporting the hypothesis, hereditary adrenal hypertrophy has been used as an indicator of a greater adaptability to stress in animals (Hessing et al., 1994; Prasolova and Os'kina, 2001), and an evolved baseline adrenal

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<sup>3</sup>INC Biomeicals, Inc., Costa Mesa, CA.

**TABLE 1. Effect of genetic-environmental interactions on plasma dopamine (DA) and corticosterone (CORT) concentrations**

Treatment	HGPS <sup>1</sup>		LGPS <sup>1</sup>	
	CORT (ng/mL)	DA (ng/mL)	CORT (ng/mL)	DA (ng/mL)
Ten-bird cages	10.4 ± 1.7 <sup>a</sup>	0.15 ± 0.09 <sup>a,*</sup>	9.2 ± 1.4 <sup>a</sup>	0.44 ± 0.16 <sup>A,*</sup>
Single-bird cages	6.0 ± 0.9 <sup>b</sup>	0.44 ± 0.15 <sup>b,*</sup>	6.1 ± 0.6 <sup>b</sup>	1.78 ± 0.41 <sup>B,*</sup>
Single:Ten-bird, %	58	293	66	405

<sup>a,b</sup>Means within a column with no common superscript were statistically different ( $P < 0.05$ ).

<sup>A,B</sup>Means within a column with no common superscript were statistically different ( $P < 0.01$ ).

<sup>\*</sup>Means between the lines were statistically different in the same treatment ( $P < 0.05$ ).

<sup>1</sup>The HGPS and LGPS lines were selected from high and low productivity and survivability, respectively.

function could be a physiological mechanism to assist the animals in coping with stressors (Moberg, 1987). In contrast, heavier AG in the LGPS hens in the 10- vs. single-bird cages indicated there is a stress-induced, rather than an inherited, adrenal hypertrophy. The stress-induced adrenal hypertrophy in LGPS pullets could be associated with their cannibalistic and pecking behavior (Freire et al., 2001), resulting in the higher mortality (Cheng et al., 2001a). Similar to the present findings, strain differences in activation of the HPA axis, including adrenal hypertrophy, associated with individual stress adaptability have been found in rodents (Dhabhar et al., 1997) and pigs (Hessing et al., 1994). Animals with heavier adrenals induced by stress have a poor coping ability to environmental stressors (Sirevaag et al., 1991; Zelena et al., 1999; Moraska et al., 2000; Suleman et al., 2000).

Present and previous results indicated that, in response to social stimuli, adrenal function is more stable in the HGPS line than in the LGPS line (Hester et al., 1996b,c; Cheng et al., 2001a). These differences could be regarded as an adapted physiological mechanism of the HGPS hens for coping with the battery cage system, including sedate and passive behavior, lower cannibalistic activity (Craig and Muir, 1996b), quicker adaptation to various stressors (Hester et al., 1996b,c), and therefore resulting in higher productivity (Craig and Muir, 1996a,b; Cheng et al., 2001b). Selection-induced changes in adrenal function have also been found in guinea pigs. Domestic cavies with less aggressive and more sociopositive behavior have less HPA and SMA responses to housing environment than wild ones (Kunzl and Sachser, 1999). In addition, in an open

field test Hessing et al. (1994) reported that adrenal responses to the conflict situation are differently regulated between pigs selected for active or passive behavior. Compared to the active pigs, the passive pigs with adrenal hypertrophy have relatively more stable responses of adrenal system. The concentrations of CORT are increased in the active pigs but not in the passive pigs, even though the latter ones have higher basal CORT levels.

### **Effect of Genetic-Social Environmental Interactions on Plasma DA Concentrations**

Plasma DA concentrations were greater in LGPS than in HGPS pullets in both single- and 10-bird cages (Table 1,  $P < 0.05$ ). This result is consistent with previous findings that LGPS hens have greater plasma DA concentrations than HGPS hens housed individually (Cheng et al., 2001b). Although higher plasma DA concentrations were found in both HGPS and LGPS lines housed in the single- vs. 10-bird cages, the levels of the latter were greater (293% vs. 405%,  $P < 0.05$  and  $0.01$ , respectively). The present study demonstrated a genetic basis for differential regulations of DA levels in response to social treatments in the present chicken lines.

The differential regulation of DA concentrations between the present chicken lines could be associated with the different activities of the SMA axis in response to different social treatments, which may reflect individual coping strategies. Compared to the HGPS line, the greater levels of DA in the LGPS line housed in both single- and 10-bird cages could be related to increased aggressive behavior,

**TABLE 2. Effect of genetic-environmental interactions on adrenal gland weight**

Treatment	HGPS <sup>1</sup>			LGPS		
	BW (g)	AGW <sup>2</sup> (mg)	AGW:BW <sup>2</sup> (% mg:g)	BW (g)	AGW (mg)	AGW:BW (% mg:g)
Ten-bird cage	1,470 ± 44	77 ± 3 <sup>*</sup>	5.2 ± 0.2 <sup>*</sup>	1,464 ± 36	69 ± 3 <sup>a,*</sup>	4.7 ± 0.2 <sup>a,*</sup>
Single-bird cage	1,461 ± 51	70 ± 5 <sup>*</sup>	4.8 ± 0.3 <sup>*</sup>	1,498 ± 56	60 ± 3 <sup>b,*</sup>	4.0 ± 0.2 <sup>b,*</sup>
Single:Ten-bird, %	99	91	92	102	87	85

<sup>a,b</sup>Means within a column with no common superscript were statistically different ( $P < 0.05$ ).

<sup>\*</sup>Means between the lines were statistically different in the same treatment ( $P < 0.05$ ).

<sup>1</sup>The HGPS and LGPS lines were selected from high and low productivity and survivability, respectively.

<sup>2</sup>AGW = absolute adrenal gland weight and AGW:BW = relative adrenal gland weight.

cannibalism, and higher mortality (Cheng et al., 2001b; Craig and Muir, 1996a,b). Similar to the current results, higher concentrations of DA were found in the brain of Japanese quail with aggressive behavior (Edens, 1987). Increased DA activity was also found in the Roman rats selected for high avoidance but not in their counterparts (Corda et al., 1997). In contrast to their counterparts, high avoidance rats have more locomotor activities that are markers of anxiety and stressful status (Corda et al., 1997). In dopamine-transporter knockout mice, hyperdopaminergia also is associated with behavioral disturbance, such as spontaneous hyperlocomotion (Spielewoy et al., 2000). In addition, the lower productivity of LGPS hens (Cheng et al., 2001a) could be related to the tonic inhibition of endogenous DA on the secretion of luteinizing hormone-releasing hormone (Contijoch et al., 1992) and luteinizing hormone (Martin et al., 1981).

Previous studies have shown that the reaction to stress is altered in animals isolated without social stressors (Sanchez et al., 1998; Haller, 2001). Consistent with these findings, the current data suggest that the single-bird cage environment is an isolated stressor to the pullets, as the plasma concentrations of DA were greater in the two lines in single-bird cages vs. 10-bird cages. In agreement with the current data, a positive correlation between enhanced activation of the DA system and increased physiological activities has been demonstrated in the chicks during social separation (Muller and Scheich, 1986). Indirect evidence also comes from Gross and Siegel's (1980) study, in which chickens housed individually were in poorer health, shown by reduced weight gain, decreased feed consumption, and increased susceptibility to *E. coli* infection compared with those in a stable group environment (nine birds/cage). Similar isolation stress reactions have also been found in rodent studies, which showed that social isolation induces changes in behavior and dopamine systems. It has been reported that a positive correlation between enhanced activities of the dopamine systems is associated with higher levels of DA (Gambardella et al., 1994; Kehoe et al., 1996) and increased sensitivity to stress (Popovic et al., 1997).

### **General Discussion of Associations of Regulation of DA and Adrenal Function in Response to Different Environments**

Differential responses between DA and adrenal function in the pullets housed in the single-bird cages are consistent with the hypothesis that different stress systems could differ in their response patterns toward stress (Schrader and Ladewig, 1999). Hens housed individually in cages may be at a lower level of social stress than those housed in a group (Craig and Muir, 1996a,b; Gross and Siegel, 1980; Hester et al., 1996a,b,c), but stress reactivity is evident. Stress reactions could be similar to the findings in mammalian studies, including alterations of monoamine systems (Frances and Monier, 1991; Sanchez et al., 1998; Cheng et al., 2001b) and exhibition of abnormal behavior (Sufka and Weed, 1994). These results may suggest that

the monoamine system including DA is more sensitive than others, such as CORT, in response to isolation. Similar to the present results, social isolation without increased or even decreased CORT concentrations has been reported in isolated animals (Benton and Brain, 1981; Armario et al., 1983; Hennessy, 1997; Haller, 2001). Gross and Siegel (1985) also reported that there are similar responses to nonsocial stressors in the chickens selected for high or low CORT response to social stress. The present and previous results indicate stress responses of animals are species, strain, and stressor specific.

The differential associations among adrenal function, behavioral exhibition, and physical parameters in response to a crowded environment between the selected lines are consistent with the hypothesis that, in poultry, population differences exist in response to various stressors (Gross and Siegel, 1985; Mench and Ottinger, 1991), and social interaction among chickens can be a serious source of stress (Gross and Siegel, 1985). The present data support that the HGPS hens, but not the LGPS hens, could maintain peacefully the social rank order developed after recaging through their lifespan (Hester et al., 1996b,c; Muir and Craig, 1998).

In conclusion, the present study provides evidence that genetic differences in chickens' productivity and behavioral styles are associated with hereditary plasticity of the neuroendocrine system, including the HPA and SMA axes. The genetic differences for differential regulation of adrenal function and plasma DA concentrations affect the present chicken lines in their response to different social environments. These differences under prolonged social stress conditions may magnify the line's unique features in physical parameters, behavioral patterns, resistance to stressors, and immunity.

### **ACKNOWLEDGMENTS**

The authors thank J. Johnson and K. Scott for assistance in collecting samples, and D. C. Lay, Jr., and R. Freire for assistance in preparing the manuscript.

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