# BREEDING AND GENETICS

# Endotoxin Stress Responses in Chickens from Different Genetic Lines. 1. Sickness, Behavioral, and Physical Responses

H. W. Cheng,*[,1] R. Freire,† and E. A. Pajor‡

*Livestock Behavior Research Unit, USDA-ARS, West Lafayette, Indiana 47907, †Centre for Neuroscience and Animal Behaviour, School of Biological, Biomedical and Molecular Sciences, University of New England, NSW2351; and ‡Department of Animal Sciences, Purdue University, West Lafayette, Indiana 47907

**ABSTRACT** Genetic variation in response to lipopolysaccharide (LPS) challenge was studied in chicken lines divergently selected for high (HGPS) and low (LGPS) group productivity and survivability resulting from cannibalism and flightiness in colony cages and in a Dekalb XL (DXL) commercial line. Six-week-old chicks were randomly assigned to control or experimental groups and were injected intravenously with *Escherichia coli* LPS (5 mg/kg of BW) or distilled saline (control). Sickness responses were measured at 6, 12, 24, 48, and 72 h following injection (n = 10 at each point in time for each line). Although LPS induced widespread sickness symptoms in all of the treated chicks, the reactions were in a genotypic- and phenotypic-specific manner. Compared with LGPS and DXL chicks, HGPS chicks had acute, transient behavioral and physical changes with less effect on BW gain, organ development, and core temperature, which were in the order HGPS < DXL < LGPS. The effects of heritable factors and LPS challenge on the differential responses among the present lines may reflect each line's unique adaptability to stress and resistance to infection and inflammation. The results suggested that the present chicken lines may provide a valuable animal model for investigating the effects of genetic-environmental interactions on the behavioral and physiological homeostasis in response to stress and disease.

(*Key words*: genetic selection, lipopolysaccharide, sickness behavior, physical index, chicken)

2004 Poultry Science 83:707–715

## INTRODUCTION

Poultry production has been greatly expanding to meet increased demands of the growing human population. The selection of chickens in the intensive production system has resulted in remarkable increases in production efficiency, but some production practices may subject animals to unintended stress, such as a crowded social environment for laying hens housed in battery cages. This situation may affect animal welfare as well as increase stress-related diseases. One solution to these problems is to improve the animal's ability to cope with the intensive production environment through genetic adaptation. Due to inherent differences in the capability to maintain behavioral and physiological homeostasis in response to disease and stressful stimuli, selective breeding of chickens for genetic or phenotypic features associated with specific behavioral and physiological characteristics has become a major tool to combat these problems and improve animal well-being (Buchenauer, 1990; Siegel and Dunnington, 1997).

A genetic basis of differentially regulated behavior and physiological performance in response to stress has been found in chickens from White Leghorn lines selected for high (HGPS) or low (LGPS) group productivity and survivability in colony cages (Craig and Muir, 1996a; Muir, 1996; Muir and Craig, 1998; Cheng et al., 2001a; Freire et al., 2001). The HGPS line (previously named KGB, kinder gentler bird) showed improved rates of lay, survival, and feather score as well as reduced cannibalism and flightiness compared with hens from a commercial line, Dekalb XL (DXL), and reversed selected LGPS line (previously named MBB, mean bad birds) (Craig and Muir, 1996a,b; Cheng et al., 2001a). Compared with hens from LGPS and DXL lines, HGPS hens also had better and faster adaptation to various stressors such as social stress, handling and transport stress, and cold and heat stimuli (Hester et al., 1996a, b, c). In addition, HGPS hens displayed greater cell-mediated immunity with a higher ratio of $CD4^+$:$CD8^+$ T cells, whereas LGPS hens exhibited eosino-



Received for publication August 12, 2003.
Accepted for publication December 4, 2003.

[1]To whom correspondence should be addressed: hwcheng@purdue.edu.

**Abbreviation Key:** DXL = Dekalb, a commercial chicken line; HGPS = hens with high group productivity and survivability; IL = interleukin; LGPS = hens with low group productivity and survivability; LPS = lipopolysaccharide.

philia and heterophilia and had a greater ratio of heterophil:lymphocyte (Cheng et al., 2001b). Both eosinophilia and heterophil:lymphocyte ratio have been used as stress indicators in animals, including chickens (Gross and Siegel, 1983; Maxwell and Burns, 1986; Maxwell, 1993; Woolaston et al., 1996). Collectively, genetic selection has resulted in lines with significantly different phenotypes, each of which has unique characteristics in physical indices, behavior, and resistance to stressors. It is important to identify the cellular mechanisms underlying these differences in the present lines in response to stress, which should provide important information for developing management strategies to minimize the impact of environmental stressors and disease on animal growth and well-being.

Endotoxin lipopolysaccharide (LPS), an integral component of the outer membrane of gram-negative bacteria, is frequently used as an objective and reliable quantitative indicator to test an animal's susceptibility to harmful pathogens and capability to adapt to immune stressors. Peripherally and centrally administered LPS cause sickness symptoms including fever, reduction of weight gain and food intake, and changes in animal behavior, including birds (Johnson et al., 1993; Xie et al., 2000; Koutsos and Klasing, 2001). In mammals, LPS-induced acute phase response is species and individual dependent (Leininger et al., 1998). Although recent findings suggest that birds show many similar response patterns to LPS-immune challenge as mammals (Parmentier et al., 1998; Webel et al., 1998; Xie et al., 2000; Koutsos and Klasing 2001), it is still unclear how genetic factors affect chickens' performance in response to environmental and disease stressors. Examination of LPS-induced sickness symptoms in animal strains, each with unique characteristics in behavioral, physical, and physiological parameters, will be useful in understanding the effects of genetic variation on animals' stress response and disease resistance.

In the present study, the effect of LPS injection on chickens' acute phase response was examined in the aforementioned divergently selected lines and a commercial line. The hypothesis that susceptibility to pathogen infection and its effect on production in chickens is genotype specific was also examined.

## MATERIALS AND METHODS

### Genetic Lines

One-day-old chicks (n = 180) used in the current study were obtained from the Purdue Poultry Farm and were from the ninth generation of the HGPS and LGPS lines and a commercial DXL line. The differences in productivity, survivability, and behavioral responses to stressors of these lines have been reported previously (Muir and Craig, 1998; Cheng et al., 2001a; Freire et al., 2001). The chicks were housed in starter batteries, at 5 chicks per cage, with ad libitum access to water and commercial feed that met the requirements suggested by the National Research Council (1994). Light schedule was 24 h constantly for the first 3 d, then a 12L:12D cycle was started at 0800 h until the end of the study.

Chicken care guidelines were in strict accordance with the rules and regulations set by the Federation of Animal Science Societies (Craig et al., 1999). The 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.

### Immune Treatment

Immunological stress was induced using LPS (*Escherichia coli*, serotype 0111:B4).[2] Sterile saline was used as the reconstitute solution because it does not induce hyper- or hypothermia in birds, including chickens (Laurin and Klasing, 1987; Koutsos and Klasing, 2001). At 6 wk of age, the chicks were randomly divided into saline control and experimental groups; then each group was divided into 5 subgroups of 10 chicks each (n = 10 at each point in time for each line). Experimental chicks were injected intravenously with 0.2 mL of sterile saline reconstituted LPS at an approximate dose of 5.0 mg/kg of BW. A previous study has showed that use of *E. coli* LPS to induce clinical symptoms in chicks is safe even at a dose of about 500 mg/kg BW (Adler and DaMassa, 1979), which is 100 times greater than the dose used in the study. The saline control chicks were handled the same as the experimental chicks except that they were injected intravenously with 0.2 mL of sterile saline. To avoid any errors that might result from time of injection, treatment was applied to one chick of each paired group (experimental and control) of each line by repeating the cycle of HGPS, LGPS, and DXL until the end.

### Body and Organ Weight

The chicks were terminated by cervical dislocation at 6, 12, 24, 48, and 72 h after injection. Body weight was measured immediately following removal of chicks from their home cages. The LPS-induced changes of BW were expressed as a percentage of the mean BW of experimental chicks:sham control chicks. Heart, spleen, and liver were also dissected from each chick terminated at each point in time. The weight of each organ was measured immediately following dissection and was represented as a relative change to BW, i.e., (organ weight:BW) $\times$ 100.

### Body Temperature

Cloacal temperature was measured at 6, 12, 24, 48, and 72 h after LPS injection with a 4600 Series Precision thermometer[3] with a 1-mm pediatric probe that was inserted 5 cm beyond the vent. To avoid any errors that might be caused by chance and time of measurement, the

[2]Sigma Chemical Co., St. Louis, MO.
[3]Yellow Springs Instruments, Inc. Dayton, OH.

temperature was taken from one chick of each paired group (experimental and control) of each line by repeating the cycle of HGPS, LGPS, and DXL until the end.

### Behavioral Observation

Immediately following LPS injection, chicks from each cage were marked on either the left or right wing with 1 of 5 colors (red, blue, green, yellow, or orange) to allow identification of individual birds. The behavior of each chick was recorded by direct observation in the hour prior to termination. An experimenter moved approximately 2 m from the front of the cage and remained still. After 1 min to allow the birds to settle, behavior was recorded by instantaneous scan sampling for each chick every minute for 10 min before the experimenter moved to another location. Behavior was recorded as 1 of 5 categories: feeding, drinking, moving, standing, and sitting, with an emphasis on LPS-induced sickness behaviors characterized by reduced food intake and decreased social activities (Dantzer et al., 1998).

### Statistical Analysis

Physical data (BW, organ weight, and body temperature) were compared by analysis of variance using the general linear model procedure of the SAS software (SAS Institute, 1992). Behavioral data were transformed into relative change of each particular activity (observed percentage of time in the experimental chicks:percentage of time engaged in the sham controls). The data were compared by two-way ANOVA to examine genetic differences in behavioral responses to LPS injection. When a significant difference was obtained ($P < 0.05$), the differences between treatments within a single time frame were tested using post-hoc paired *t*-tests.

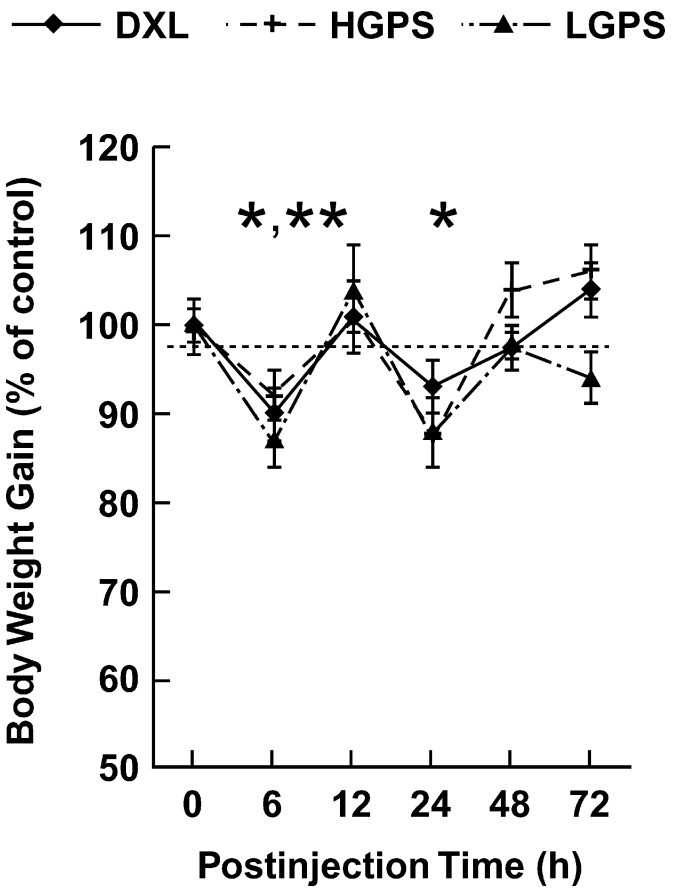


**FIGURE 1.** Differential regulation of BW gains in different chicken lines following lipopolysaccharide (LPS) intravenous injection. Hens had been selected for high (HGPS) or low (LGPS) group productivity and survivability, and the Dekalb (DXL) was a commercial chicken line. Compared with BW from their respective controls, the BW gain was significantly reduced in DXL chicks at 6 h postinjection ($P < 0.05$); in LGPS chicks at 6 and 24 h postinjection ($P < 0.05$), and in HGPS chicks at 24 h postinjection ($P < 0.01$). *$P < 0.05$; **$P < 0.01$ (n = 10 at each period in time for each line).

## RESULTS AND DISCUSSION

### LPS-Induced Changes in Body Weight and Organ Weight in Different Chicken Lines

The present study demonstrated that LPS-induced immune stress differently affected chickens' growth among the HGPS, LGPS, and DXL lines. In DXL chicks, change in BW gain exhibited a biphasic pattern, i.e., a greater reduction of BW gain at 6 h postinjection ($P < 0.05$) and a tendency for reduction of BW gain at 24 h postinjection ($P = 0.08$), followed by a full recovery at 48 h postinjection (Figure 1). Compared DXL chicks, LGPS chicks, but not HGPS chicks, had a similar biphasic pattern of reduction of BW gain in response to LPS immune challenge. In LGPS chicks, reduction of BW gain was greater at both 6 h and 24 h postinjection ($P < 0.05$) and did not reach a positive BW gain at 72 h postinjection. In contrast, HGPS chicks did not have a reduction of BW gain until 24 h postinjection ($P < 0.05$), which was followed by a complete recovery at 48 h postinjection ($P < 0.05$) and a positive BW gain from 48 h to 72 h postinjection.

The interactions of genetic-LPS challenge on chicks' growth among the present chicken lines were also found in their organ development. Compared with each line's respective controls, spleen weight increased in DXL chicks at 48 h postinjection and reached a peak at 72 h postinjection ($P < 0.05$ and $P < 0.01$, respectively, Figure 2a), whereas LPS-induced increases in spleen weight were not detected in HGPS or LGPS chicks until 72 h after injection ($P < 0.05$). The LPS injection also resulted in a differential change of liver weight among the lines (Figure 2b). Compared with each line's respective controls, the LPS-induced increase in the liver weight was found only in LGPS chicks from 12 to 48 h postinjection ($P < 0.01$ and $P < 0.05$, respectively, Figure 2b). There were no changes in the heart weight in DXL or HGPS chicks at any time measured ($P > 0.05$), whereas LGPS chicks had an increased heart weight during the entire treatment period, with a peak at 72 h postinjection ($P < 0.05$ and $P < 0.01$, respectively, Figure 2c). The LPS-induced increase

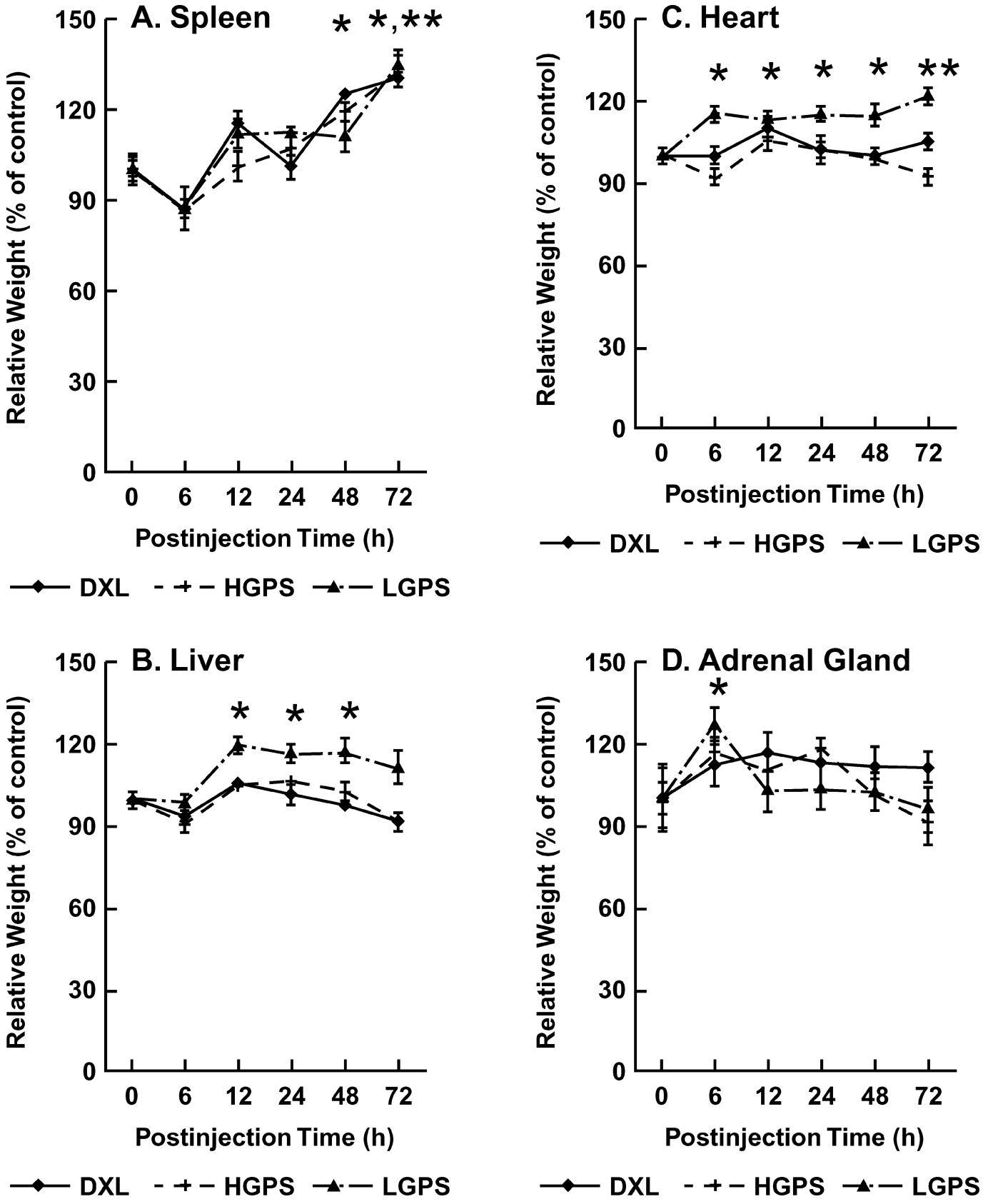


**FIGURE 2.** Differential regulation of organ weight in different chicken lines following lipopolysaccharide (LPS) intravenous injection. Hens had been selected for high (HGPS) or low (LGPS) group productivity and survivability, and the Dekalb (DXL) was a commercial chicken line. Compared with their respective controls, spleen weight (A) was significantly increased in DXL chicks at 48 h ($P < 0.05$) and 72 h ($P < 0.01$) postinjection and in HGPS and LGPS chicks at 72 h postinjection ($P < 0.05$, respectively). Increased liver weight (B), heart weight (C), and adrenal gland weight (D) were found in LGPS chicks but not in HGPS or DXL chicks from 12 to 48 h postinjection ($P < 0.05$), 6 to 72 h ($P < 0.05$ and $P < 0.01$, respectively), or at 6 h ($P < 0.05$) postinjection, respectively. *$P < 0.05$; **$P < 0.01$ (n = 10 at each period in time for each line).

in adrenal weight was found in LGPS chicks at 6 h postinjection ($P < 0.05$ and $P > 0.05$, respectively, Figure 2d), whereas adrenal weight was unchanged in HGPS and DXL chicks during the entire observed period ($P > 0.05$).

The present results showed that, compared with both LGPS and DXL chicks, HGPS chicks had a delayed and transient reduction of BW gain and mild changes in organ development in response to LPS challenge (Figures 1 and 2). The data confirmed that the acute toxicity of LPS induced sickness symptoms including reduction of BW gain and changes in organ development in animals, but the effect of LPS on chickens was strain and time dependent. Similar to the present results, a genetic basis of different effects of LPS injection on BW gain was also reported by Parmentier et al. (1998). In their study, they found that although LPS injection induced an acute, transient reduction of BW weight in all of the chicken lines, chickens selected for high antibody response to SRBC had a higher percentage of BW gain than chickens selected for low antibody response to SRBC and a randombred control line.

The reason for the differing regulation of growth performance in the present lines could be related to each line's unique characteristics in response to stress. Previous studies showed that, compared with LGPS and DXL chickens, HGPS chickens had a better fast coping response to various stressors, such as social stress, handling and transport stress, and cold and heat stimulations (Hester et al., 1996a, b, c; Cheng et al., 2001a,b, 2002). The HGPS chickens, compared with LGPS and DXL chickens, also had a stable neuroendocrine homeostasis in response to social stress, which could be related to their higher resistance to LPS stress (Cheng et al., 2002, 2003). In agreement with this hypothesis, Quan et al. (2001) and Carobrez et al. (2002) reported that impaired coping capability to social stress increases the susceptibility to LPS challenge in rodents and caused long-term consequences on animal well-being.

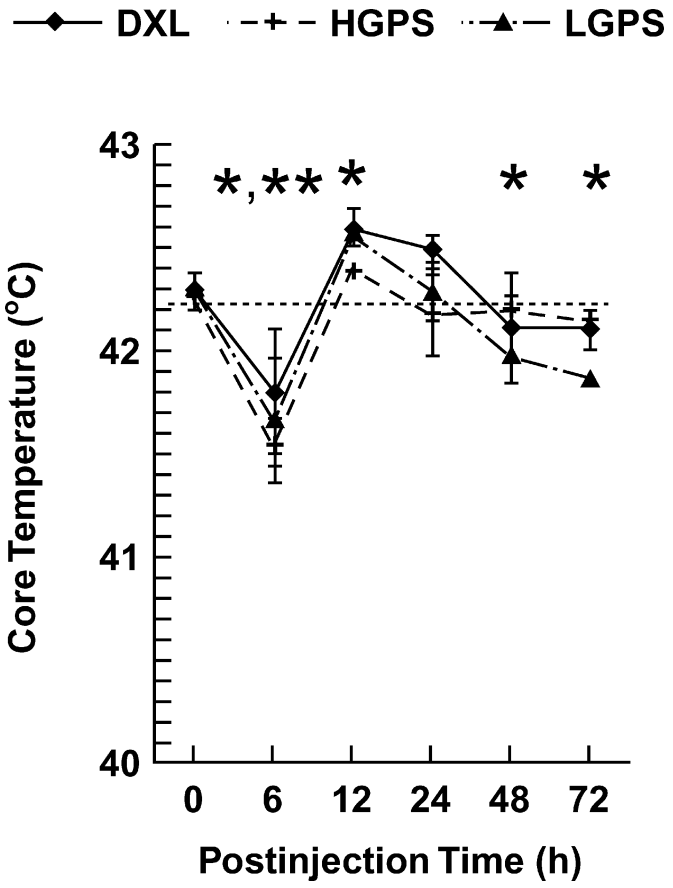


**FIGURE 3.** Differential regulation of core temperature in different chicken lines following lipopolysaccharide (LPS) intravenous injection. Hens had been selected for high (HGPS) or low (LPGS) group productivity and survivability, and the Dekalb (DXL) was a commercial chicken line. Compared with their respective controls, LPS injection resulted in hypothermia in all of the treated chicks at 6 h postinjection, but the greatest reduction of core temperature was found in HGPS chicks ($P < 0.05$ and $P < 0.01$, respectively). By 12 h postinjection, DXL and LGPS chicks, but not HGPS chicks, had hyperthermia. Core temperature returned to control levels at 24 h postinjection in DXL and HGPS chicks, whereas LGPS chicks had secondary hypothermia from 48 to 72 h after injection ($P < 0.05$). *$P < 0.05$ and **$P < 0.01$ (n = 10 at each period in time for each line).

## *LPS-Induced Changes of Body Temperature in Different Chicken Lines*

The present study demonstrated that LPS-induced changes of core temperature (cloacal temperature) in chicks were strain and time dependent. Compared with the respective controls for each line, LPS injection resulted in hypothermia in all of the treated chicks at 6 h postinjection regardless of the strain (Figure 3), but the greatest hypothermia was found in HGPS chicks (HGPS < LGPS < DXL, $P < 0.001$, $P < 0.01$, and $P < 0.05$, respectively). At 12 h postinjection, LPS induced a significant hyperthermia in both DXL and LGPS chicks ($P < 0.05$ and $P < 0.01$, respectively) but not in HGPS chicks ($P = 0.09$). From 12 to 72 h postinjection, compared with their respective controls, the core temperature returned to normal in both DXL and HGPS chicks ($P > 0.05$), while LGPS chicks had a secondary hypothermia from 48 to 72 h postinjection ($P < 0.01$ and $P < 0.05$, respectively).

The present results showed that LPS injection induces changes in core temperatures of chickens regardless of strain. However, each strain had a unique pattern of regulating core temperature in response to LPS immune stress (Figure 3). The HGPS chicks had transient monophasic hypothermia, the DXL chicks had a biphasic response showing an initial hypothermia followed by hyperthermia, and the LGPS chicks had a triphasic response showing an initial hypothermia, then hyperthermia, followed by a longer-lasting secondary hypothermia. Similar to the current results, previous studies found that LPS induced different fever responses in birds, such as a monophasic hypothermia in chicks (Smith et al., 1978) and a biphasic response, i.e., an initial phase of hypothermia followed by a fever response, in chickens (Rotiroti et al., 1981), Japanese quail (Koutsos and Klasing, 2001), and pigeons (Nomoto, 1996). The LPS-induced biphasic and triphasic responses were also found in rats (Derijk and Berken-

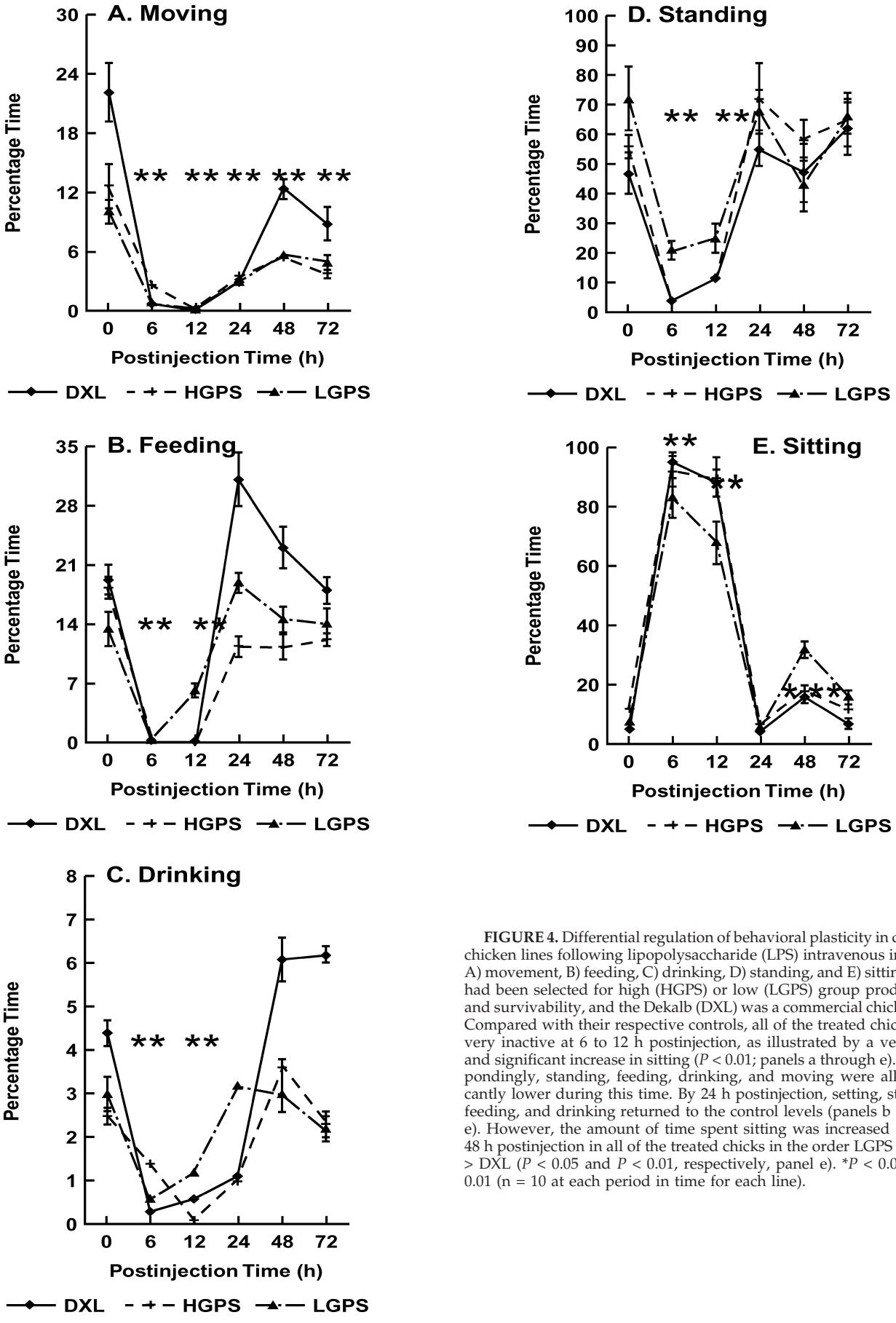


**FIGURE 4.** Differential regulation of behavioral plasticity in different chicken lines following lipopolysaccharide (LPS) intravenous injection; A) movement, B) feeding, C) drinking, D) standing, and E) sitting. Hens had been selected for high (HGPS) or low (LGPS) group productivity and survivability, and the Dekalb (DXL) was a commercial chicken line. Compared with their respective controls, all of the treated chicks were very inactive at 6 to 12 h postinjection, as illustrated by a very large and significant increase in sitting ($P < 0.01$; panels a through e). Correspondingly, standing, feeding, drinking, and moving were all significantly lower during this time. By 24 h postinjection, setting, standing, feeding, and drinking returned to the control levels (panels b through e). However, the amount of time spent sitting was increased again at 48 h postinjection in all of the treated chicks in the order LGPS > HGPS > DXL ($P < 0.05$ and $P < 0.01$, respectively, panel e). *$P < 0.05$; **$P < 0.01$ (n = 10 at each period in time for each line).

bosch, 1994; Romanovsky et al., 1996) and mice (Kozak et al., 1994). The genetic bases of the different responses to the LPS immune stress between animals are likely to constitute an intrinsic characteristic of the animals' unique febrile response and could result from their capability to resist stress. The hypothesis is supported by the findings from the previous studies in which it was reported that psychological stress itself can induce an increase in core temperature, "psychogenic fever," in humans and animals (Oka et al., 2001).

The mechanisms of differential regulation of core temperature between the present lines could be related to the unique pattern of each line in coping with stressors, such as the capability of behavioral and physiological plasticity including changes in the neuroendocrine and immune systems (Cheng et al., 2001a,b, 2002, 2003). A parallel study showed that LPS injection induced changes of interleukin (IL), such as IL-1 mRNA expressions, in the liver of all of the LPS-treated chicks (Eicher and Cheng, 2003), but LGPS chicks had a heavier liver than both DXL and HGPS chicks at 12 and 48 h postinjection, during which period LGPS chicks suffered from secondary hypothermia. These results may suggest that, in response to endotoxin challenge, the liver functions of LGPS chicks were increased and might have secreted a greater amount of IL-1 protein. The hypothesis agrees with the finding that the liver is a major source of IL in endotoxemia. The LPS-induced increase in the liver's metabolic function and increase in the release of acute phase proteins and cytoleukines including IL-1 have been reported in experimental animals, including chickens (Xie et al., 2000). In addition, previous studies have reported that IL-1 functions as an endogenous pyrogen and regulates endotoxin-induced sickness symptoms in mammals (Plata-Salaman et al., 1998; Leon, 2002). Although a similar correlation of LPS-induced changes of IL-1 concentration and sickness behavior has been found in birds (Macari et al., 1993; Xie et al., 2000) and a bird's IL-1, such as IL-1 $\beta$, is homologous to that of mammals, further studies are needed to determine whether IL are regulated differently at protein levels, i.e., synthesis, secretion, or degradation among the present lines.

## LPS-Induced Change of Behavior in the Different Chicken Lines

The majority of significant behavioral differences between LPS and saline control groups were observed from 6 to 12 h postinjection. During this period, chicks were very inactive, as illustrated by a very large and significant increase in sitting ($P < 0.001$, Figure 4). Correspondingly, standing, feeding, drinking, and moving were all significantly lower during this time compared with control chicks. By 24 h postinjection, sitting, standing, feeding, and drinking returned to control levels (Figure 4, b through e). However, the amount of time spent sitting was increased again at 48 h postinjection in all of the treated chicks, with a time in the order LGPS > HGPS > DXL (Figure 4e). The increase in sitting in LGPS chicks could be related to their secondary peak of hypothermia, which started at 48 h postinjection (Figure 2). Interestingly, the amount of time that chicks spent moving was suppressed in all LPS-injected groups and had not returned to control levels even after 72 h postinjection, suggesting that there may still have been some mild effect from the LPS injection (Figure 4a).

Chicks from different genetic lines were found not to differ in their sickness behaviors in response to LPS injection (data not shown). Although the finding suggested that behavioral responses to LPS challenge have not been altered through selection, the data support the theory that the acute cachectin nature of LPS and sickness behavior in animals has a common phyletic origin.

## General Summary

The LPS injection induced a series of sickness symptoms in the infected individuals at both behavioral and clinical levels, but the reactions were in a genotypic- and phenotypic-specific manner. Compared with both LGPS and DXL chicks, HGPS chicks had acute, transient behavioral and physical changes with less effect on BW gain and organ development. These results suggested that genetic selection for productivity and survivability may also have altered the mechanisms controlling the animals' stress response including LPS challenge. This hypothesis is in agreement with the previous findings that the genetic selection for one indicator could result in changes in other characteristics in animals including chickens. For instance, chickens selected for their high level of plasma corticosterone, compared with a reversely selected line, greatly resisted *E. coli* challenge (Gross and Siegel, 1975). Bayyari et al. (1997) also reported that genetic selection from increased body weight and egg production in turkeys affected their immune and physiological responses.

Although the reason for the differential regulation of behavioral and physical changes in the selected lines remains unclear, previous studies in mammals have shown that different components of sickness symptoms could be mediated by different mechanisms. For example, following LPS injection, different IL are reactivated differently in the peripheral and central cytokine compartments, and each IL has unique functions in regulating acute phase response and sickness behavior (Dantzer, 2001). The same cellular mechanisms could be involved in regulating LPS responses in the present selected lines, since there is evidence that the functions of the avian neuroendocrine and immune systems that control stress responses are analogous to those in mammals (Harvey et al., 1984; Johnson, 1998).

In conclusion, the present study provided evidence that genetic differences in chickens' productivity and behavioral styles were associated with hereditary plasticity of the behavioral and physiological homeostasis in response to LPS challenge. The LPS-induced alterations in behavioral and physical measurements were found in all 3 chicken lines, but the most pronounced changes were found in the LGPS line. The results demonstrated that,

in chickens as in mammals, the cellular mechanisms regulating the response to LPS challenge are species and strain dependent. The differential responses between the present lines are consistent with the hypothesis that, in poultry, population differences exist in response to various stressors, and LPS challenge can be a useful indicator to evaluate the efficacy of immunity and capability to adapt infection in poultry. The present chicken lines may provide a new animal model for studying the behavior and physiology of infection and inflammation in poultry.

## ACKNOWLEDGMENTS

The authors thank Pete Singleton, Sophia Wilcom, Kim McMunn, and Mike Toscano for their assistance in collecting sample, and D. C. Lay Jr. for his assistance in preparing the manuscript.

## REFERENCES

Adler, H. E., and A. J. DaMassa. 1979. Toxicity of endotoxin to chicks. Avian Dis. 23:174–178.

Bayyari, G. R., W. E. Huff, N. C. Rath, J. M. Balog, L. A. Newberry, J. D. Villines, J. K. Skeeles, N. B. Anthony, and K. E. Nestor. 1997. Effect of the genetic selection of turkeys for increased body weight and egg production on immune and physiological responses. Poult. Sci. 76:289–296.

Buchenauer, D. 1990. Genetic behavioral aspects of agricultural animals. Dtsch. Tierarztl. Wochenschr. 97:247–9.

Carobrez, S. G., O. C. Gasparotto, B. Buwalda, and B. Bohus. 2002. Long-term consequences of social stress on corticosterone and IL-1beta levels in endotoxin-challenged rats. Physiol. Behav. 76:99–105.

Cheng, H. W., G. Dillworth, P. Singleton, Y. Chen, and W. M. Muir. 2001a. Effect of Genetic Selection for Productivity and Longevity on Blood Concentrations of Serotonin, Catecholamines, and Corticosterone of laying hens. Poult. Sci. 80:1278–1285.

Cheng, H. W., S. D. Eicher, Y. Chen, P. Singleton, and W. M. Muir. 2001b. Effect of genetic selection for group productivity and longevity on immunological and hematological parameters of chickens. Poult. Sci. 80:1079–1086.

Cheng, H. W., P. Singleton, and W. M. Muir. 2002. Social stress in laying hens: differential dopamine and corticosterone responses after intermingling different genetic strains of chickens. Poult. Sci. 81:1265–72.

Cheng, H. W., P. Singleton, and W. M. Muir. 2003. Social stress in laying hens: differential effect of stress on plasma dopamine concentrations and adrenal function in genetically selected chickens. Poult. Sci. 82:192–8.

Craig, J. V., W. F. Dean, G. B. Havenstein, K. K. Kruger, K. E. Nestor, G. H. Purchase, P. B. Siegel, and G. L. van Wicklen. 1999. Guidelines for poultry husbandry. Pages 55–66 in Guide for the Care and Use of Agricultural Animals in Agricultural Research and Teaching. Federation of Animal Science Societies. Savoy, Illinois, USA.

Craig, J. V., and W. M. Muir. 1996a. Group selection for adaptation to multiple-hen cages: beak-related mortality, feathering, and body weight responses. Poult. Sci. 75:294–302.

Craig, J. V., and W. M. Muir. 1996b. Group selection for adaptation to multiple-hen cages: behavioral responses. Poult. Sci. 75:1145–1155.

Dantzer, R. 2001. Cytokine-induced sickness behavior: mechanism and implications. Ann. N. Y. Acad. Sci. 933:222–234.

Dantzer, R., R. M. Bluthe, G. Gheusi, S. Cremona, S. Laye, P. Parnet, and K. W. Kelley. 1998. Molecular basis of sickness behavior. Ann. N. Y. Acad. Sci. 856:132–138.

Derijk, R. H., and F. Berkenbosch. 1994. Hypothermia to endotoxin involves the cytokine tumor necrosis factor and the neuropeptide vasopressin in rats. Am. J. Physiol. 266:R9–14.

Eicher, S. D., and H. W. Cheng. 2003. Toll-like receptor 2 and acute phase cytokine responses by genetically selected chickens following an LPS challenge. J. Fed. Am. Soc. Exp. Biol. 17:C51 (Abstr.).

Freire, R., P. Singleton, Y. Chen, M. W. Muir, E. Pajor, and H. W. Cheng. 2001. The relationship between physiological parameters and behavioral response to social stress among three genetic lines of laying hens. Poult. Sci. 80(Suppl. 1):280. (Abstr.)

Gross, W. B., and P. B. Siegel. 1975. Immune response to Escherichia coli. Am. J. Vet. Res. 36:568–571.

Gross, W. B., and H. S. Siegel. 1983. Evaluation of the heterophil/lymphocyte ratio as a measure of stress in chickens. Avian Dis. 27:972–979.

Harvey, S., J. G. Phillips, A. Rees, and T. R. Hall. 1984. Stress and adrenal function. J. Exp. Zool. 232:633–645.

Hester, P. Y., W. M. Muir, and J. V. Craig. 1996a. Group selection for adaptation to multiple-hen cages: humoral immune response. Poult. Sci. 75:1315–1320.

Hester, P. Y., W. M. Muir, J. V. Craig, and J. L. Albright. 1996b. Group selection for adaptation to multiple-hen cages: production traits during heat and cold exposures. Poult. Sci. 75:1308–1314.

Hester, P. Y., W. M. Muir, J. V. Craig, and J. L. Albright. 1996c. Group selection for adaptation to multiple-hen cages: hematology and adrenal function. Poult. Sci. 75:1295–1307.

Johnson, R. W. 1998. Immune and endocrine regulation of food intake in sick animals. Domest Anim. Endocrinol. 15:309–319.

Johnson, R. W., S. E. Curtis, R. Dantzer, and K. W. Kelley. 1993. Central and peripheral prostaglandins are involved in sickness behavior in birds. Physiol. Behav. 53:127–131.

Koutsos, E. A., and K. C. Klasing. 2001. The acute phase response in Japaneses quail (*Coturnix coturnix japonica*) Comp. Biochem. Physiol. 128:255–263.

Kozak, W., C. A. Conn, and M. J. Kluger. 1994. Lipopolysaccharide induces fever and depresses locomotor activity in unrestrained mice. Am. J. Physiol. 266:R125–135.

Laurin, D. E., and K. C. Klasing. 1987. Effects of repetitive immunogen injections and fasting feeding on iron, zinc, and copper metabolism. Biol. Trace Elem. Res. 14:153–165.

Leininger, M. T., C. A. Portocarrero, C. A. Bidwell, M. E. Spurlock, J. N. Nielsen, and K. L. Houseknecht. 1998. Effect of immune challenge on different genotypes: How sick do they get? Purdue University Swine Day Report. Purdue University, Purdue, IN.

Leon, L. R., 2002. Molecular Biology of thermoregulation invited review: Cytokine regulation of fever studies using gene knockout mice. J. Appl. Physiol. 92:2648–2655.

Macari, M., R. L. Furlan, F. P. Gregorut, E. R. Secato, and J. R. Guerreiro. 1993. Effects of endotocin, interleukin-1 beta and prostaglandin injections on fever responses in broilers. Br. Poult. Sci. 34:1035–1042.

Maxwell, M. H. 1993. Avian blood leucocyte responses to stress. Worlds Poult. Sci. J. 49:34–43.

Maxwell, M. H., and R. B. Burns. 1986. Experimental stimulation of eosinophil production in the domestic fowl (*Gallus gallus domesticus*). Res. Vet. Sci. 41:14–23.

Muir, W. M. 1996. Group selection for adaptation to multiple-hen cages: selection program and direct responses. Poult. Sci. 75:447–458.

Muir, W. M., and J. V. Craig. 1998. Improving animal well-being through genetic selection. Poult. Sci. 77:1781–1788.

National Research Council. 1994. Nutrient Requirements of Poultry. 9th rev. ed. National Academy Press, Washington DC.

Nomoto, S. 1996. Diurnal variations in fever induced by intravenous LPS injection in pigeons. Pflugers Arch. 431:987–989.

Oka, T., K. Oka, and T. Hori. 2001. Mechanisms and mediators of psychological stress-induced rise in core temperature. Psychosom. Med. 63:476–486.

Parmentier, H. K., M. Walraven, and M. G. Nieuwland. 1998. Antibody responses and body weights of chicken lines selected for high and low humoral responsiveness to sheep red blood cells. 1. Effect of Escherichia coli lipopolysaccharide. Poult. Sci. 77:248–255.

Plata-Salaman, C. R., E. Peloso, and E. Satinoff. 1998. Interleukin-1 beta-induced fever in young and old Long-Evans rats. Am. J. Physiol. 275:R1633–1638.

Quan, N., R. Avitsur, J. L. Stark, L. He, M. Shah, M. Caligiuri, D. A. Padgett, P. T. Marucha, and J. F. Sheridan. 2001. Social stress increases the susceptibility to endotoxic shock. J. Neuroimmunol. 115:36–45.

Rotiroti, D., A. Foca, P. Mastroeni, D. Fumarola, and G. Nistico. 1981. Behavioural and body temperature effects of meningococcal lipopolysaccharide after intraventricular injection in adult fowls Gallus domesticus. Res. Commun. Chem. Pathol. Pharmacol. 33:395–402.

Romanasky, A. A., V. A. Kulchitsky, C. T. Simons, and N. Sugimoto. 1996. Methodology of fever research: Why are polyphasic fevers often thought to be biphasic? Am. J. Physiol. 275:R332–338.

SAS Institute. 1992. SAS User's Guide to the Statistical Analysis System. North Carolina State University, Raleigh, NC.

Siegel, P. B., and E. A. Dunnington. 1997. Genetic selection strategies—population genetics. Poult. Sci. 76:1062–5.

Smith, I. M., S. T. Licence, and R. Hill. 1978. Haematological, serological and pathological effects in chicks of one or more intravenous injections of *Salmonella gallinarum* endotoxin. Res. Vet. Sci. 24:154–160.

Webel, D. M., R. W. Johnson, and D. H. Baker. 1998. Lipopolysaccharid-induced reductions in body weight gain and feed intake do not reduce the efficiency of arginine utilization for whole-body protein accretion in the chick. Poult. Sci. 77:1893–1898.

Woolaston, R. R., P. Manueli, S. J. Eady, I. A. Barger, L. F. Le Jambre, D. J. Banks, and R. G. Windon. 1996. The value of circulating eosinophil count as a selection criteria for resistance of sheep to trichostrongyle parasites. Int. J. Parasitol. 26:123–126.

Xie, H., N. C. Rath, G. R. Huff, W. F. Huff, and J. M. Balog. 2000. Effects of *Salmonella typhimurium* lipopolysaccharide on broiler chickens. Poult. Sci. 79:33–40.