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**Abstract**: The genetic control of sickness symptoms to lipopolysaccharide (LPS) 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-wk-old chicks were randomly assigned to control or experimental groups and were injected intravenously with Escherichia coli LPS (5 mg/kg BW) or distilled saline (control). Sickness responses were measured? at 6, 12, 24, 48 and 72 h following injection. Although LPS induced widespread sickness symptoms in all of the treated chicks, the reactions were in a genotypic- and phenotypic-dependent manner. Compared to both LGPS and DXL chicks, HGPS chicks had acute, transient behavioral and physical changes. (you may want to expand the results a bit here to say exactly what we found)The effects of heritable factors and LPS immune challenge on the differential responses between the present lines are discussed, which may reflect each lines unique adaptability to stress and resistance to infection and inflammation. The results suggest 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.

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Running title: STRESS-INDUCED BEHAVIROAL AND PHYSICAL CHANGES IN CHICKENS.

**Different effects of endotoxin stress on chickens from different genetic lines: I. Sickness behavioral and physical responses**

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Abbreviation Key**:**

DXL = Dekalb, a commercial chicken line; HGPS = hens with high group productivity and survivability; IL-1 = interleukin-1; LGPS = hens with low group productivity and survivability; LPS = lipopolysaccharide; SRBC = sheep red blood cells; 5-HT = serotonin;

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**ABSTRACT** The genetic control of sickness symptoms to lipopolysaccharide (LPS) 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-wk-old chicks were randomly assigned to control or experimental groups and were injected intravenously with *Escherichia coli* LPS (5 mg/kg BW) or distilled saline (control). Sickness responses were measured? at 6, 12, 24, 48 and 72 h following injection. Although LPS induced widespread sickness symptoms in all of the treated chicks, the reactions were in a genotypic- and phenotypic-dependent manner. Compared to both LGPS and DXL chicks, HGPS chicks had acute, transient behavioral and physical changes. (you may want to expand the results a bit here to say exactly what we found)The effects of heritable factors and LPS immune challenge on the differential responses between the present lines are discussed, which may reflect each line's unique adaptability to stress and resistance to infection and inflammation. The results suggest 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)

# INTRODUCTION

Poultry production has been greatly expanding to meet increased demands of the expanding human population size. The selection of chickens to the intensive production system has resulted in remarkable increases in production efficiency, but many production practices affect well – being? . This situation creates an unacceptable production risk that affects both animals' psychological and physiological well-being as well as increases stress-related disease, which can led to decrease productivity, increase physical and emotional suffering and death of birds. One solution to these problems is to improve the animal's ability to cope with the intensive 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, Craig & Swanson, 1994; Mench & Duncan, 1998; Newman, 1994; Siegel, 1989; Siegel & 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 (Cheng et al., 2001a; Craig & Muir, 1996a, Muir, 1996; Muir & Craig, 1998; Freire & Cheng, 2001). Group productivity was based on an average rate of lay whereas survivability was based on days of survival. Chickens were not beak-trimmed and high light intensity was used to provide conditions that allowed expression of aggressive behavior with resulting stress and productivity impacts (Craig et al., 1999; Craig & Muir, 1996 a, b). Under these housing

conditions, HGPS line (previously named KGB, Kinder and Gentler Bird) showed an improved rate of lay, survival and feather score as well as reduced cannibalism and flightiness compared to hens from a commercial line, Dekalb XL (DXL), and reversed selected LGPS line (previously named MBB, Bad and Mean Birds) (Cheng et al., 2001a; Craig & Muir, 1996a,b). Compared to hens from LGPS line and DXL line, HGPS hens also had better and faster adaptation to various stressors such as social stress, handling and transport stress, cold and heat stimulations (Hester et al., 1996a, b, c). In addition, HGPS hens displayed greater cell-mediated immunity with a higher ratio of $CD4^+$:$CD8^+$ T cells, while LGPS hens exhibited eosinophilia and heterophilia and had a greater ratio of heterophil:lymphocyte (H/L) in single-hen cages (Cheng et al., 2001b). Both eosinophilia and H/L have been used as stress indicators in animals, including chickens (Gross and Siegel, 1983; Maxwell & Burns, 1983; Maxwell, 1993; Woolaston et al., 1996; Hohenhaus et al., 1998). Collectively, genetic selection has resulted in lines with significantly different phenotypes, each of which has unique characteristics in physical indexes, behavior and resistance to stressors. It is important to identify the cellular mechanisms underlying these differences in the present lines in response to infection?, which is critical issue for developing management strategies to minimize the impact of environmental stressors and disease on animal growth, well-being.

The 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 stressors. Peripheral or central administrated LPS causes sickness symptoms including fever, reduction of weight grain and food intake as well as changes of behavior in animals

including birds (Johnson et al., 1993; Xie et al., 2000; Koutsos & Klasing., 2001). In mammals, LPS-induced acute phase response is in a species (gene)- and individual feature (phenotype)-dependent manner (Leininger et al., 1998). Although the findings from the recent studies suggest that birds show many similar patterns of responses to LPS immune challenge as mammals (Nakamura et al., 1998; Parmentier et al., 1998; Webel et al., 1998; Xie et al., 2000; Koutsos & Klasing 2001), it is still unclear how the genetic factors affect chickens' performance in response to environmental and disease stressors, which would be greatly facilitated by examination of LPS-induced sickness symptoms in animal models with unique genetic characteristics in regulation of behavioral and physical patterns.

In the present study, the effect of LPS injection on chicken acute phase response was examined in the divergently selected lines and a commercial line. We also examined the hypothesis that susceptible to pathogen infections and its effect on production in chickens are genetic-dependent.

## MATERIALS AND METHODS

### *Genetic Lines*

One-day-old chicks used in this study were obtained from the Purdue Poultry Farm, which were from the 9th generation of the HGPS and LGPS lines and the commercial DXL line. The differences in productivity and survivability of these lines have been reported previously (Muir, 1998; Cheng et al., 2001a). The chicks were housed in starter batteries with *ad libitum* access to water and commercial feed that met NRC (1994) requirements. Light schedule was 24-h constantly from day 1 to day 3, followed by a 12-h light/12-h dark light cycle until the end of the study.

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.

***Immune Treatment***

Immunological stress was induced using LPS (*Escherichia Coli*, serotype 0111:B4, Sigma[1]). Sterile saline was used as reconstitute solution since it does not induce hyper- or hypothermia in birds including chickens (Koutsos & Klasing, 2001; Laurin & Klasing, 1987). At 6 wk of age, the chicks were randomly divided into sham control and experimental groups, then each group were divided into 5 subgroups of 10 chicks each. Experimental chicks were injected intravenously with 0.2 ml sterile saline reconstituted LPS at an approximately dose of 5.0mg/kg BW. A previous study has showed that use of *E coli* LPS to induce clinical symptoms in chicks is save even at a large dose, such as at about 500 mg/kg BW, which dose is 100 x greater than the dose used in the study (Adler & DaMassa, 1979). The sham control chicks were handled as the same as the experimental chicks except that they were injected intravenously with 0.2 ml sterile saline. To avoid any errors may be caused by consequence and time of injection, treatment was applied to one chick of each paired groups (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 at 6, 12, 24, 48 and 72 h after injection. Body weight was measured immediately following the chicks removed from their home cages. LPS-induced

[1] Sigma, St. Louis, Missouri, USA

changes of BW were presented as a percentage of the mean BW of experimental chicks/sham control chicks. Chicks were killed by cervical dislocation, and selected organs, including heart, spleen and liver, were dissected without fat. The weight of each organ was measured immediately following dissection and was represented as a relative change to BW, i.e., (organ weight/BW) x 100.

***Body Temperature***

Cloacal temperature was measured at 6, 12, 24, 48 and 72 h after LPS injection with a 4600 Series Precision thermometer with a 1-mm pediatric probe that was inserted 5 cm beyond vent (YSI Inc, Yellow Springs, OH[2]). To avoid any errors may be caused by consequence and time of measurement, temperature was taken from one chick of each paired groups (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 either on the left or right wing with one of five colors (red, blue, green, yellow and orange) to allow identification of individual birds. Behavior of each chick was recorded by direct observation in the hour prior to termination (08:00am). An experimenter moved to approximately 2 m from the front of the cage and remained still. After one minute to allow the birds to settle, behavior was recorded by instantaneous scan sampling for each chick every minute for 15 minutes before the experimenter moved to another location. Behavior was recorded as one of five categories: feeding, drinking, moving, standing and sitting.

[2] Yellow Spring Springs Instruments, Inc., Dayton, OH 45440-3605

### *Statistical Analysis*

Changes in BW were calculated as: percentage of the average BW of treated chicks/sham control chicks X 100. All data were analyzed by two-way ANOVA with SAS® GMLGLM? procedure (SAS institute, Inc., Raleigh, NC). 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). Relative data was compared by two-way ANOVA to examine genetic differences in behavioral responses to LPS injection. When a significant ($P<0.05$) obtained, differences between treatments within a single time point were tested using post-hoc paired t-tests.

## RESULTS AND DISCUSSION

### *LPS-induced different changes in body weight and organ weight in different chicken lines*

The present study demonstrated that LPS-induced immune stress affected growth of chickens differently among the present reversely selected HGPS and LGPS lines and DXL commercial line. In DXL chicks, loss of BW grain exhibited a biphasic pattern, i.e., a greater loss of BW gain at 6 h post-injection ($P<0.05$) and a tendency for reduction of BW gain at 24 h post-injection ($P=0.08$), followed by a full recovery at 48 h post-injection (Figure 1). Compared to 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, loss of BW gain was greater at both 6 h and 24 h post-injection ($P<0.05$) and did not reach a positive BW gain at 72 h post-injection (Figure 1). In contrast, HGPS chicks did not had a loss of BW gain until 24 h post-injection ($P<0.05$), followed by a completive recovery at 48 h post-injection ($P>0.05$), and reached a plateau in weight gain from 48 h to 72 h post-injection (Figure 1).

The different effects of the interactions of genetic-LPS immune challenge on chicks' growth between the present chicken lines were also found in their organ development. Compared to their respective controls, spleen weight was increased in DXL chicks at 48 h post-injection and reached a peak at 72 h post-injection ($P<0.05$ and 0.01 respectively, Figure 2a); while LPS-induced an increase in spleen weight did not detect in both HGPS and LGPS chicks until 72 h after injection ($P<0.05$). LPS injection also resulted in a different alteration of liver weight between the lines (Figure 2b). Compared to their respective controls, LPS-induced an increase in the liver weight was found only in LGPS chicks from 12 to 48 h post-injection ($P<0.01$ and 0.05, respectively, Figure 2b). There were no changes in the heart weight in both DXL and HGPS chicks at all of the time points ($P<0.05$,) while LGPS chicks had an increased heart weight during the entire treatment period, with a peak at 72 h post-injection ($P<0.05$ and 0.01, respectively, Figure 2c). LPS-induced increase in adrenal weight was found in LGPS chicks but not in HGPS or DXL chicks at 6 h post-injection during the entire treatment period ($P<0.05$ and $P>0.05$, respectively, Figure 2d).

The present results showed that, compared to 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 immune challenge (Figure 1and 2). The data confirm that acute toxicity nature of LPS inducing sickness symptoms, such as reduction of BW gain and changes of organ developments, in animals, but the effect of LPS on chickens is in a stain- and time-dependent manner. Similar to our results, a genetic basis of LPS-induced different responses of BW gain has been found in rodents (Fraifeld et al., 1998; Neveu et al., 1998) and chickens (Fraifeld t al., 1998; Parmentier et al., 1998). Fraifeld and Kaplanski (1998) reported that different species and strains of rodents (rats and mice) and chickens exhibited

rather specific sickness response to LPS injection. A similar result 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 used in their study, chickens selected for high antibody response to sheep red blood cells (SRBC) had a higher percentage BW gain than chickens selected for low antibody response to SRBC and a random bred 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 to LGPS and DXL chickens, HGPS chickens had a better and fast coping to various stressors, such as social stress, handling and transport stress, cold and heart stimulations (Hester et al., 1996 a,b,c; Cheng et al., 2001a,b; 2002). HGPS chickens, compared to LGPS and DXL chickens, also had a quite stable neuroendocrine homeostasis in response to social stress, which could be related to their higher resistant to LPS stress (Cheng et al., 2002, 2003). Similar to our findings, Quan et al. (2001) and Carobrez et al. (2002) reported that social stress increases the susceptibility to LPS immune challenge in rodents, and causes long-term consequences on animal well-being.

***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 is strain- and time-dependent. Compared to their respective controls, LPS resulted in hypothermia in all of the treated chicks at 6 h post-injection at regardless of the strains (Figure 3) but, compared to DXL and LGPS chicks, the greatest hypothermia was found in HGPS chicks ($P<0.05$, 0.01 and 0.001, respectively). At 12 h post-injection, LPS induced a significant hyperthermia in both DXL and LGPS chicks ($P<0.05$

and 0.01, respectively) but not in HGPS chicks (P=0.09). At the time period from 12 to 72 h post-injection, compared to controls, the core temperature was recovery in both DXL and HGPS chicks (P>0.05) while LGPS chicks had a second hypothermia from 48 to 72 h post-injection (P<0.01 and 0.05, respectively, Figure 3).

The present results showed that LPS injection induces changes of chickens' core temperature regardless of their strain. However, each strain has a unique pattern of regulating core temperature in response to LPS immune stress (Figure 3). HGPS chicks had transient monophasic hypothermia; DXL chicks had a biphasic fever showing an initial hypothermia followed by hyperthermia, and LGPS chicks had a triphasic fever which an initial hypothermia, then hyperthermia, and followed by a longer-lasting second hyperthermia. There different responses to the LPS immune stress between the lines are likely to constitute an intrinsic characteristic of its unique febrile response and could result from its capability of resistant to stress (Hester et al., 1996 a,b,c; Cheng et al., 2001, 2002). Previous studies have reported that psychological stress itself can induces an increase in core temperature, "psychogenic fever", in humans and animals (Oka et al., 2001).

Similar to our results, LPS-induced different fever responses have been found in animals including birds, which is genotypic- and phenotypic-dependent. In birds, LPS-induced hypothermia, a monophasic response, has been found in chicks (Smith et al., 1978), and a biphasic response, i.e., an initial phase of hypothermia followed by a fever response, was found in chickens {if you want o give latin names, suggest you do so for all these animals- personally I would just call it chicken!}(Rotiroti et al., 1981), Japanese quail (Koutsos & Klasing, 2001) and pigeon (Nomoto, 1996). LPS-induced biphasic and triphasic response were also found in rats (Derijk et al., 1996; Romanovsky et al., 1996; 1998) and

mice (Kozak, 1994). In contrast, a recent study reported that LPS induces a monophasic fever response in broiler chickens (Xie et al., 2000). The different febrile responses found between the present and previous studies could be related to using of different strain of chickens, layer vs. broiler; different age of chickens, 6-wk-old vs. 3-wk-old; different post-injection time, 6 h vs. 3 h; and different type of LPS serology, *E. coli* vs. *S. typhimuriums*. The hypothesis is in agreement with the previous findings that LPS induced sickness responses in chickens are affected by multiple factors, which are animal species-, strain-, age-, LPS serologic stains and doses- as well as post-injection time-dependent (Rotiroti et al., 1981; Rimler 1984; Jones et al., 1983; Sunwoo et al., 1996; Fraifeld & Kaplanski, 1998; Parmentier et al., 1998; Dogan et al., 2000; Leshchinsky & Klasing, 2001).

The mechanism(s) of different regulations of core temperature between the present lines could be related to each line's unique pattern in coping to stressors, such as the capability of behavioral and physiological plasticity including changes of the neuroendocrine system (Cheng et al., 2001a,b 2002, 2003). In the previous studies, we have reported that LGPS chickens have higher concentrations of serotonin (5-HT) than HGPS chickens, which may be related to the longer-lasting hypothermia found in LGPS chicks. 5-HT has functions in regulating body temperature by increasing of animals' sensitization to LPS immune challenge (Hayley et al., 2001; Oka et al., 2001). LPS-induced increases in 5-HT concentrations and its catabolism have been found in rodents (MohanKumar et al., 1999), which were coincided with the LPS-induced acute phase reaction including biphasic fever and tail flick-induced the initial hyperalgesia and hypoalgesia in the second phase of the acute phase reaction (Koulchitsky et al., 2000). In addition, hypothermia can be induced by pharmacological activity the 5-HT 1A receptors with agonists such as buspirone (Blier et al.,

2002) or can be blocked by knockout of 5-HT7 receptor genes (Hedlund et al., 2003), which further supports that 5-HT is involved in hypothermic reaction. Furthermore, changes of 5-HT concentrations affect regulations of cytoleukines, such as interleukin 1 (IL-1) (Wilcox et al., 1994; Imeri et al., 1999), interleukin 6 (Ito et al., 200) and tumor necrosis factor alpha (Cho et al., 1999). The interactions between 5-HT and interleukins such as IL-1 may be involved in the different regulation of LPS-induced sickness symptoms between the present lines. One of our parallel study showed that LPS injection induced changes of IL-1 mRNA expressions in the liver of the treated chicks (Echei and Cheng, 2003), but LGPS chicks had a heavier liver than both DXL and HGPS chicks at 12 and 48 h post-injection, as the same time as LGPS chicks had the second hypothermia, which suggests that, in response to endotoxin challenge, the liver function of LGPS chicks was increased and may secret a greater amount of IL-1 protein. The hypothesis is in agreement with the findings that the liver is a major source of interleukins in endotoxemia, and LPS induced increase in liver's metabolic function and increase in releasing acute phase proteins and cytoleukines including IL-1 have been reported in experimental animals (Chensue et al., 1991; Luster et al., 1994; Brouwer et al., 1995; Yoshioka et al., 1998; Kmiec 2001) including chickens (Xie et al., 2000). IL-1 has functions as an endogenous pyrogen in response to endotoxin-induced sickness symptoms in mammals (Plata-Salaman et al., 1998; Inui, 2001; Leon, 2002). Although a similar correlation of LPS-induced changes of IL-1 concentration and sickness behavior has been found in birds (Macari M. et al., 1993; Xie et al., 2000) and bird's IL-1, such as IL-1 beta, is homology to that of mammals, further studies are needed to determine whether interleukins are regulated differently at protein levels, i.e., synthesis, secretion and/or degradation between the present lines.

***LPS-induced change of behavior in different chicken lines***

The majority of significant behavioral differences between LPS and saline groups were observed from 6 to 12 h post-injection. During this period, chicks were very inactive, as illustrated by very large and significant increase in sitting (approximately 70%, $P<0.001$, Figure 4a-e). Correspondingly, standing, feeding, drinking and moving were all significantly lower during this time compared to saline-injected chicks. By 24 h post injection, sitting, standing, feeding and drinking returned to similar levels to controls (Figure 4 b-e). However, the amount of time spent sitting was increased again at 48 h post injection in all of the treated chicks, with a time length in the order LGPS > HGPS > DXL (Figure 4e). The increase in sitting in LGPS chicks could be related to their second perk of hypothermia which started at 48 h post-injection (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 post-injection (Figure 4a).

Chicks displayed clear signs of sickness at both 6 and 12 h post injection, with most behavioral patterns returning to control levels after 24 h. However, chicks did not resume control levels of moving even at 72 h post-injection, suggesting that there may still have been some mild effect from the LPS injection (Figure 4a). (this repeats the previous paragraph a bit- could you combine it?)

Sickness behavior in the genetic lines was not found to differ in their response to LPS injection. Although the finding suggests that behavioral responses to LPS immune challenge have not been altered through genetic selection, the data support to the theory that acute cachectin nature of LPS and that sickness behavior in animals has a common phyletic origin.

***General summary***

LPS induces a whole set of sickness symptoms in the infected individual at both behavioral and clinical levels, but the reactions are in a genotypic- and phenotypic-dependent manner. Different components of sickness symptoms could be mediated by different mechanisms. For example, following LPS injection, different interleukins are reactivated differently in the peripheral and central cytokine compartments, and each interleukin has unique functions in regulating acute phase response and sickness behavior (Dantzer, 2001; Dantzer et al., 1998), which may explain the dissociation between mechanisms controlling the behavioral and physiological responses shown in mammals (Aubert, 1999; Dantzer, 2001; Dantzer et al., 1998; Delrue et al., 1994; Johnson, 2002). There may be the same reasons that are related to LPS-induced different changes in behavior and growth performance between the present lines.

Genetic selection may result in different alterations of the mechanisms controlling LPS-induced sickness symptoms among animals that exhibit different reactions in response to the immune challenge. The hypothesis is in agreement with the previous findings that genetic selection for one indicator could result in change of other indicators in animals (Hessing et al., 1994; Jones et al., 1994; Nestor et al., 2000) including chickens (Gross & Siegel, 1975, 1980; Siegel et al., 1993; Yonash et al., 1996; Bayyari et al., 1997; Yunis et al., 2002). For instance, chickens selected for high level of plasma corticosterone, compared to reverse selected line, greatly resisted *E. coli* challenge (Gross & Siegel, 1975). Although the latter ones had greater body weight gain and produced antibody earlier and longer with higher antibody titers. Interactions between genetic selection-infection also found in the

chickens selected for high and low antibody titers to SRBC. Compared to the line selected for a lack of ability to produce antibody titers to SRBC, the line with higher antibody titers exhibited stronger antibody reaction to *Newcastle* disease, *Mycoplasam gallisepticum, Eimeria necatrix*, and feather mites but less resistant to *E. coli* and *Staphylococcus aureus* infection (Gross et al., 1980).

In conclusion, the present study provides the evidence that genetic differences in chickens' productivity and behavioral styles are associated with hereditary plasticity of the behavioral and physiological homeostasis in response to LPS immune challenge. LPS-induced alterations in behavioral and physical changes were found in all of the three chicken lines, but most pronounced changes were found in LGPS line. The results demonstrated that, in chickens, as in mammals, the cellular mechanisms regulating the response to LPS immune challenge are species-, strain-, and time-dependent. The differential responses between the present lines are consistent with the hypothesis that, in poultry, population differences exist in response to various stressors (Gross & Siegel, 1985; Mench & Ottinger, 1991), and LPS immune 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 behavior and physiology of infection and inflammation in poultry.

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**FIGURE LEGENDS**:

Figure 1. Differential regulation of BW gain in different chicken lines following LPS intravenous injection. Following an intravenous injection of LPS, compared to BW from their respective controls, DXL chicks had a significant loss BW gain at 6 h ($P<0.05$) and a tendency for loss BW gain at 24 h ($P=0.08$); LGPS chicks had significant loss BW gain at both 6 and 24 h post-injection ($P<0.05$) while HGPS chicks had a significant loss BW at 24 h post-injection ($P<0.01$). HGPS and LGPS hens were selected for high or low group productivity and survivability; DXL, a commercial line. *, Statistical significant at $P<0.05$ or 0.01. n=10/time point/line.

Figure 2. Differential regulation of organ weight in different chicken lines following LPS intravenous injection. 2a Spleen. compared to their respective controls, spleen weight was increased in DXL chicks at 48 h ($p<0.05$) and reach a peak at 72 h($P<0.01$) post-injection while spleen weight of both HGPS and LGPS chicks did not reach a significant increase until 72 h post-injection ($P<0.05$, respectively); 2b Liver. LPS-induced increase in liver weight in LGPS chicks but not in both HGPS and DXL chicks from 12 to 48 h post-injection ($P<0.05$ and $P>0.05$, respectively); 2c Heart. LGPS chicks but not HGPS and DXL chicks had an increased heart weight during the entire treatment period ($P<0.05$ and 0.01, respectively); and 2d adrenal gland. LPS-injection induced increase in adrenal weight was found in LGPS chicks but not in HGPS and DXl chicks at 6 h post-injection

during the entire treatment period (P<0.05 and >0.05, respectively). HGPS and LGPS hens were selected for high or low group productivity and survivability; DXL, a commercial line. *, Statistical significant at P<0.05 or 0.01. n=10/time point/line.

Figure 3. Differential regulation of core temperature in different chicken lines following LPS intravenous injection. Compared to their respective controls, LPS intravenous injection resulted in hypothermia in all of the treated chicks at 6 h post-injection but the greatest reduction of core temperature was found in HGPS chicks (P<0.05 and <0.01, respectively); At 12 h post-injection, both DXL and LGPS chicks but not HGPS chicks had hyperthermia; and during 24 to 72 h post-injection, core temperature returned to the control levels at 24 h in both DXL and HGPS chick but LGPS chicks had a second hypothermia from 48 to 72 h. HGPS and LGPS hens were selected for high or low group productivity and survivability; DXL, a commercial line. *, Statistical significant at P<0.05 or 0.01. n=10/time point/line.

Figure 4. Differential regulation of behavioral plasticity in different chicken lines following LPS intravenous injection, a) movement, b) feeding, c) drinking, d) standing and c) sitting. Compared to their respective controls, at 6 to 12 h post-injection, all of the treated chicks were very inactive, as illustrated by very large and significant increase in sitting (P<0.01, Figure 4a-e). Correspondingly, standing, feeding, drinking and moving were all

significantly lower during this time compared to control chicks. By 24 h post-injection, setting, standing, feeding and drinking returned to similar levels to controls (Figure 4 b-e). However, the amount of time spent sitting was increased again at 48 h post injection in all of the treated chicks, with a time length in the order LGPS > HGPS > DXL (Figure 4e). Interestingly, the amount of time that chicks spent moving did not returned to control levels even at 72 h post-injection (Figure 4a). HGPS and LGPS hens were selected for high or low group productivity and survivability; DXL, a commercial line. *, Statistical significant at $P<0.05$ or 0.01. n=10/time point/line.