

Effect of early life thermal conditioning and immune challenge on thermotolerance and humoral immune competence in adult laying hens

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ABSTRACT Effects of early life experience with climatic (heat) and hygienic [lipopolysaccharide (LPS)] stress on adaptability to the same stressors in later life were studied in laying hens. Chicks were exposed to 37°C for 24 h at d 5 of age (n = 12) or were i.v.-administered once with 1 mg/kg of BW of LPS at 6 wk of age (n = 12), whereas a control group was reared under standard conditions receiving a placebo treatment of PBS (n = 36). At 24 wk of age, hens treated in early life were reexposed to the same stressor. Early life control hens were exposed to heat stress (n = 12), i.v.-administered with LPS (n = 12), or not exposed (n = 12). To evaluate improvement of adaptability, effects of climatic and hygienic stress on performance, humoral immune competence, and endocrine responsiveness were investigated in hens with early life experience to

the stressors and hens only exposed to the stressors in later life. Early life heat exposure did not affect performance, immune, and endocrine parameters. Treatment × time interactions were found for level of antibody (Ab) binding to LPS and keyhole limpet hemocyanin (KLH) after LPS administration, indicating that hens with early life LPS experience differed in response level (Ab binding to LPS) and response pattern (Ab binding to LPS and KLH) compared with hens administered with LPS only at adult age. Our data suggest that early life heat stress exposure did not affect adaptability of laying hens to heat stress in later life. However, early life LPS exposure affected kinetics and magnitude of Ab levels binding to LPS and KLH, indicating that early life LPS exposure can enhance the status of immune reactivity or induce a higher sensitivity to LPS.

Key words: early life experience, heat stress, antibody, lipopolysaccharide, performance

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INTRODUCTION

Animals can be conditioned to an expected change in the environment. For instance, broiler chickens can be physiologically modulated by thermal conditioning in early life to improve heat stress tolerance in later life (Arjona et al., 1990; Yahav and Hurwitz, 1996; Yahav and Plavnik, 1999; De Basilio et al., 2001). The main idea in the thermal conditioning process is to manipulate immature mechanisms of temperature regulation in early life, enabling chickens to cope, within certain limits, with acute exposure to unexpected heat spells in later life (Yahav and Hurwitz, 1996; Yahav and Mc-

Murtry, 2001). In broiler chickens, thermal conditioning to heat at an early age resulted in reduced weight gain during the first week of life, followed by an accelerated growth (Yahav and Plavnik, 1999; Yahav and McMurtry, 2001), improved thermotolerance, and reduced mortality when reexposed to heat in later life (Arjona et al., 1990; Yahav and Hurwitz, 1996; Yahav and Plavnik, 1999; De Basilio et al., 2001). Early thermal conditioning seems to be one of the most promising methods to improve the adaptability of broiler chickens to heat stress (Lin et al., 2006).

Such a conditioning process may also be used to prepare animals to hygienic conditions. Gram-negative bacteria are ubiquitous in the environment of poultry and, in particular, inhalation of their endotoxin has been recognized as an important factor in the prevalence of respiratory diseases (Zucker et al., 2000). One of the major endotoxins in the poultry environment is lipopolysaccharide (LPS). The endotoxin LPS is often

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used as a model antigen to study the susceptibility of an animal to nonspecific components of microbiological pathogens and capability to react to microbial stressors (Ulevitch and Tobias, 1999). In this respect, it is noteworthy that chickens become refractory to repeated LPS administrations, accompanied by a minimal decreasing effect on performance characteristics (e.g., feed intake and growth; Korver et al., 1998; Parmentier et al., 2006). Repeated LPS administrations seem to stimulate adaptation through an unknown mechanism and, therefore, might improve the adaptability to this stressor.

Early life experience with climatic conditions has been studied in broilers, but not in laying hens. Early life experience with hygienic conditions has potential, but effects on basic adaptation parameters, such as hen-day egg production and feed intake, have not been studied before in laying hens. Besides, little is known about the effects of early life experience with hygienic conditions on development of (natural) humoral immunity. In the present study, we hypothesized on the basis of the mentioned literature as reviewed above that early life experiences with climatic and hygienic stress would improve the adaptability of laying hens to these stressors. To test this hypothesis, laying hens were exposed to heat stress or LPS administration in early life and were reexposed to similar stressors as adult hens during early lay. These stressors have also been used in previous studies (Star et al., 2007, 2008a,b), in which adult hens were exposed to single or combined climatic stress (heat) and hygienic stress (LPS) during early lay. In the present study, the focus will be on improvement of adaptability of adult laying hens (e.g., the effect of early life experience on responses in later life), rather than the difference between climatic and hygienic stress responses. To evaluate the improvement of adaptability, effects of climatic and hygienic stress on performance (e.g., feed intake, BW, hen-day egg production, egg weight, and clutch length), humoral immune competence, and endocrine responsiveness were investigated in hens with early life experience to the stressors and hens only exposed to the stressors in later life.

MATERIALS AND METHODS

Experimental Design

In this study, 60 purebred layer chicks (Rhode Island Red; line B1) were used. At day of hatch, chicks were obtained from a hatchery (Hendrix Genetics, Boxmeer, the Netherlands) and placed in a climate respiration chamber (control chamber) at Wageningen University. At d 1 after hatch, chicks were randomly assigned to 1 of 5 treatment groups (Table 1):

- 1) Heat early life and adult experience: heat stress at 5 d of age and 24 wk of age.
- 2) Heat adult experience: heat stress at 24 wk of age only.
- 3) LPS early life and adult experience: LPS administration at 6 and 24 wk of age.
- 4) LPS adult experience: LPS administration at 24 wk of age only.
- 5) Control: no heat stress and no LPS administration at young and older age.

Heat Early Life Experience. At d 1, twelve randomly chosen chicks were housed in a smaller climate chamber for 1 wk (d 1 to 7 after hatch). At d 5 after hatch, chicks in the small climate respiration chamber were exposed to heat stress. Within approximately 1 h, temperature in the small chamber increased from 32 to 37°C and was maintained at 37°C during the following 24 h. In the control chamber, temperature was maintained at 32°C according to the normal rearing temperature at this age. After 1 wk in the climate chambers, all chicks were moved and housed in the rearing facility, where they were reared under standard rearing conditions.

LPS Early Life Experience. In the rearing facility at the age of 6 wk, twelve chicks were i.v.-administered with 1 mg/kg of BW of *Escherichia coli* LPS (serotype O55:B5, Sigma Chemical Co., St. Louis, MO). The remaining chicks received a placebo treatment of PBS.

Adult Experience. At 22 wk of age, 59 hens were moved to 2 identical large climate respiration chambers (1 hen was removed from the experiment because of

Table 1. Experimental design

Treatment group	Temperature (°C) ¹		LPS ²		Birds (n) ³
	Early life (5 d of age)	Adult (24 wk of age)	Early life (6 wk of age)	Adult (24 wk of age)	
Heat: early life and adult	Yes	Yes	No	No	12
Heat: adult	No	Yes	No	No	12
LPS: early life and adult	No	No	Yes	Yes	11
LPS: adult	No	No	No	Yes	12
Control	No	No	No	No	12

¹Temperature treatment at 5 d of age for 24 h (early life) and at 24 wk of age for 22 d (adult). Early life heat treatment was 37°C (yes) and 32°C for the untreated chicks (no). Adult heat treatment was 32°C (yes) and 21°C for the untreated laying hens (no).

²Administration of *Escherichia coli* lipopolysaccharide (LPS; i.v., 1 mg/kg of BW) at 6 wk (early life) and 24 wk of age (adult). Yes = injected with LPS; no = injected with PBS.

³Number of birds at the start of the adaptation and experimental period (22 and 24 wk of age).

egg production problems), where they were individually housed in battery cages (45 cm height × 40 cm depth × 24 cm width). After an adaptation period of 12 d (temperature maintained at 21°C), laying hens treated in early life were reexposed to the same stressor [i.e., exposed to heat stress (heat early life and adult experience, n = 12) or i.v.-administered once with 1 mg/kg of BW of LPS (LPS early life and adult experience, n = 11)]. Hens that were not exposed to either heat stress or LPS at early age received 1 of the 2 stressors at 24 wk of age only [i.e., heat stress (heat adult experience, n = 12) or i.v.-administered with LPS (LPS adult experience, n = 12)]. The remaining laying hens did not receive one of the stressors at early or adult age (control, n = 12).

Laying hens of the treatment groups “heat early life and adult experience” and “heat adult experience” were exposed to heat in one of the climate chambers at 24 wk of age (start of the experiment, d 0). Within approximately 1 h, temperature in this chamber was increased from 21 to 32°C and was maintained at 32°C during the following 22 d.

Laying hens of the treatment groups “LPS early life and adult experience,” “LPS adult experience,” and control were housed in the other chamber where temperature was maintained at 21°C. Laying hens of the treatment groups “LPS early life and adult experience” and “LPS adult experience” were i.v.-administered with 1 mg/kg of BW of *E. coli* LPS at d 1 of the experiment. The control group received a placebo treatment of PBS.

After 22 d, temperature in the heat stress chamber was decreased to 21°C. This was the end of the experiment. Each performance, immune, and endocrine parameter was established in each of the individual hens only at adult age (between 22 and 27 wk of age). The Institutional Animal Care and Use Committee of Wageningen University approved the experimental protocol.

Housing and Feed

At day of hatch, chicks were placed in a climate respiration chamber where temperature was set at 34°C and was gradually reduced by 0.5°C until 31°C at the end of the first week, except for the chicks housed in the small climate chamber that were exposed to heat stress at d 5 after hatch. Light was provided for 24 h at the first day and for 23 h during d 2 to 7. Thereafter, all chicks were reared under standard conditions in the rearing facility. In the rearing facility, chicks were housed in cages, where temperature was reduced by 1°C every 3 d until 21°C was reached at d 36. Thereafter, temperature was kept at 21°C. The light period was reduced to 19 h after the first week, and thereafter, until 16 wk of age, the light period was gradually reduced by 1 h every 2 wk, and from 16 until 18 wk of age by 1 h weekly until hens were kept under a 9L:15D light scheme. Thereafter, the

light period was increased by 1 h per week until hens were kept under a 15L:9D light scheme. During the experimental period (from 24 to 28 wk of age), hens were kept under a 15L:9D light scheme.

Chicks were fed a rearing phase 1 diet (202 g/kg of CP, 45 g/kg of crude fiber, and 11.1 MJ of ME/kg) until the age of 13 wk and a rearing phase 2 diet (176 g/kg of CP, 46 g/kg of crude fiber, and 12.1 MJ of ME/kg) until 16 wk of age. Thereafter, hens were fed a standard commercial laying phase 1 diet (155 g/kg of CP, 34 g/kg of crude fiber, and 11.8 MJ of ME/kg). Feed and water were available ad libitum throughout the experiment.

The hens received routine vaccinations against Marek's disease (d 1), Newcastle disease (d 10, 31, and 103), infectious bronchitis (d 1, 76, and 103), infectious bursal disease (d 21), fowl pox (d 56), infectious laryngotracheitis (d 85), and avian encephalomyelitis (d 94). Beak trimming was not performed.

Performance

From 22 wk of age until the end of the experiment at 27 wk of age, feed intake and egg number were recorded daily. Body weight was measured at d -5, 2, 8, 16, and 22 relative to the start of heat stress at 24 wk of age. Body weight development in time was calculated in relation to BW measured at d 5 before the start of heat stress. Egg weight was recorded weekly at d -3, 4, 11, and 18 relative to the start of heat stress. Mortality was registered. No data before 22 wk of age were collected.

Immune and Endocrine Parameters

Blood samples for plasma and serum were collected from the wing vein of all adult laying hens at d -5, 2, 5, 8, 16, and 22 after the start of heat stress. After sampling, blood was centrifuged and both plasma and serum were stored at -20°C until further processing. Plasma samples were used to analyze antibodies (Ab) and corticosterone, and serum samples were used to analyze hemolytic complement activity and mannan-binding lectin (MBL) concentration.

Humoral Immune Response to LPS and Keyhole Limpet Hemocyanin. Antibody levels binding to LPS and natural Ab levels binding to keyhole limpet hemocyanin (KLH) were determined in individual samples by an indirect ELISA procedure at d -5, 2, 5, 8, 16, and 22 relative to the start of heat stress. Flat-bottomed 96-well medium-binding ELISA plates were coated with either 4 µg/mL of *E. coli* LPS or 1 µg/mL of KLH (MP Biomedicals Inc., Aurora, OH). After subsequent washing, plates were incubated with plasma (diluted 1:40, 1:160, 1:640, and 1:2,560 for LPS and KLH). Binding of Ab to LPS and KLH antigen was visualized using a 1:20,000 diluted rabbit anti-chicken IgG_{H+L} labeled with peroxidase (RACH/IgG_{H+L}/PO; Nordic, Tilburg, the Netherlands). After washing, substrate (tetrameth-

ylbenzidine and 0.05% H₂O₂) was added, and 10 min later, the reaction was stopped with 2.5 N H₂SO₄. Extinctions were measured with a microplate reader (Multiskan, Labsystems, Helsinki, Finland) at a wavelength of 450 nm. Levels (titers) were expressed as log₂ values of the dilutions that gave an extinction closest to 50% of E_{max}, where E_{max} represents the highest mean extinction of a standard positive plasma present on each flat-bottomed ELISA plate.

Hemolytic Complement Assay. Activity of the classical complement pathway (CPW) and alternative complement pathway (APW) was determined in individual serum samples collected at d -5, 2, 5, 8, 16, and 22 relative to the start of heat stress. The hemolytic complement assay was performed according to the method described by Demey et al. (1993). Briefly, appropriate buffers were prepared. The buffer solution for CPW was prepared by adding MgCl₂ (1 mmol/L) and CaCl₂ (0.15 mmol/L) to Veronal-buffered saline (Cambrex Bio Science, Walkersville Inc., Walkersville, MD). The buffer solution for APW was prepared by adding MgCl₂ (5 mmol/L) and ethylene glycol tetraacetate (16 mmol/L) to Veronal-buffered saline.

The assay was performed in flat-bottomed 96-well microtiter plates. Sera were diluted serially in the appropriate buffers and incubated with sensitized (72202, Hemolysin, bioMérieux, Marcy l'Etoile, France) sheep erythrocytes or bovine erythrocytes prepared by standard methods and used as a 1% cell suspension to measure CPW or APW, respectively. During 1.5 h of incubation, plates were shaken every 30 min. Results (the amount of light scattering by erythrocytes upon lysis) were read in a microplate reader (Multiskan, Labsystems) at a wavelength of 690 nm. Readings were transformed by a log-log equation (Von Krogh, 1916), and the hemolytic titer was expressed as the titer that lyses 50% of the red blood cells (U/mL).

MBL Complement Assay. Activity of the MBL was determined in individual serum samples by an indirect ELISA procedure at d -5, 2, 5, 8, 16, and 22 after the start of heat stress. The MBL assay was performed according to the method described by Norup and Juul-Madsen (2007). Briefly, flat-bottomed 96-well microtiter plates (Maxisorp, Nunc, Roskilde, Denmark) were coated with 5 µg/mL of anti-chicken MBL Ab (HYB 182-01, Statens Serum Institute, Copenhagen, Denmark). After washing, residual protein-binding sites were blocked by 200 µL of 0.5% (vol/vol) Tween 20 in 10 mM Tris, 140 mM NaCl, for 33 min. The plates were then incubated with serum (diluted 1:610) for 2 h. After another washing step, plates were incubated with 1 µg/mL of biotinylated mouse anti-chicken MBL (HYB 182-01) for 45 min, followed by a 30-min incubation with 1:13,000 diluted horseradish peroxidase-conjugated streptavidin (P0397, Dako, Glostrup, Denmark). Finally, the presence of horseradish peroxidase was detected by adding 100 µL of substrate (tetramethylbenzidine). After 27

min at 26°C, color development was stopped with 100 µL of 1 M H₂SO₄ and determined by reading the absorbance at 450 nm. The MBL activity was expressed in micrograms per milliliter of serum.

Corticosterone. Corticosterone levels were determined in individual plasma samples by using a sensitive and highly specific commercial RIA kit (IDS Inc., Boldon, UK; Malheiros et al., 2003) at d -5, 2, and 5 after the start of heat stress. Before performing the assay, plasma samples were heated at 80°C for 10 min to inactivate corticosterone-binding proteins. Corticosterone concentrations were expressed in nanograms per milliliter of plasma.

Statistical Analysis

Differences between “early life and adult experience” and “adult experience” to either heat stress or LPS administration were established.

Differences in feed intake and hen-day egg production were analyzed by a 1-way ANOVA for the effect of treatment (“early life and adult experience” vs. “adult experience”). Feed intake and hen-day egg production were, after primary analyses of the daily measurements, divided in 3 periods: P1 is the adaptation period (d -11 to 0), P2 is the first week after exposure (d 1 to 7), and P3 is during the last 2 wk of exposure, in which mainly heat treatment influenced the parameters (d 8 to 22). Statistical analyses were done for each period, but no statistical analyses were done among periods.

Differences in BW development, egg weight, corticosterone, and each of the immune parameters were analyzed by a 2-way ANOVA for the effect of treatment (“early life and adult experience” vs. “adult experience”), time, and their interactions by a repeated measurement procedure using a hen nested within treatment option.

Correlations between feed intake, hen-day egg production, and egg weight before the start of the experimental period were analyzed by Pearson product-moment correlation. Data were not corrected for the effect of early life treatment.

All analyses were carried out using SAS (SAS Institute, 2004). The PROC GLM procedure was used to analyze the 1-way ANOVA, the PROC MIXED procedure was used to analyze the 2-way ANOVA with a repeated measurement procedure, and the PROC CORR procedure was used to analyze correlations. Mean differences between treatments were tested with Bonferroni's test. Effects were considered significant at $P < 0.05$.

RESULTS

Differences between “early life and adult experience” and “adult experience” to either heat stress or LPS administration were established. Data of the control

Table 2. Effect of early life exposure to heat stress on performance, immune, and endocrine parameters in later life¹

Parameter		Heat treatment			<i>P</i> -value ²		
		Control	Early life and adult	Adult	Treatment	Time	Treatment × time
Number of birds		12	12	12			
Feed intake ³ (g/d)	P1	79.0	82.4	83.7	0.8412		
	P2	95.0	52.4	48.6	0.5247		
	P3	103.5	65.2	70.7	0.3196		
Egg production ³ (%)	P1	59.0	70.1	71.5	0.9141		
	P2	89.3	54.8	50.0	0.6532		
	P3	90.0	60.6	57.2	0.7698		
BW gain ⁴ (g)		-9	-108	-148	0.3224	<0.0001	0.8416
Egg weight (g)		52.7	50.0	50.9	0.5011	<0.0001	0.3924
Complement (CH50 ⁶ value) (μg/mL)	CPW ⁵	522	438	455	0.7536	0.0003	0.6424
	APW ⁷	210	219	150	0.0233	0.0184	0.1904
	MBL ⁸	14.0	17.6	20.6	0.3771	<0.0001	0.9165
Antibody titer	LPS ⁹	2.6	3.0	2.8	0.5771	<0.0001	0.4185
	KLH ¹⁰	4.7	4.5	4.2	0.3262	<0.0001	0.1952
Corticosterone (ng/mL)		2.7	2.3	3.1	0.0101	0.0322	0.9592

¹Treatment “early life and adult” are laying hens exposed to heat stress (37°C) at 5 d of age and repeatedly exposed to heat stress (32°C) at 24 wk of age for 22 d, whereas treatment “adult” are laying hens only exposed to heat stress (32°C) at 24 wk of age for 22 d.

²Differences between “early life and adult experience” and “adult experience.” Data of the control group were not statistically compared due to the interest in differences between early life and adult experience.

³Data on feed intake and hen-day egg production were divided into 3 periods: P1 is the adaptation period (d -11 to 0), P2 is the first week of the experimental period (d 1 to 7), and P3 is the second and third week of the experimental period (d 8 to 22).

⁴The BW development in time was calculated compared with BW at d 5 before the start of heat stress.

⁵CPW = classical complement pathway.

⁶CH50 = titer that lyses 50% of the red blood cells.

⁷APW = alternative complement pathway.

⁸MBL = mannan-binding lectin.

⁹LPS = lipopolysaccharide.

¹⁰KLH = keyhole limpet hemocyanin.

group are given (Tables 2 and 3) but not statistically compared due to the interest in differences between early life and adult experience.

Heat Experience

No differences were found for BW development in time, feed intake, hen-day egg production, egg weight, level of Ab binding to LPS and KLH, and complement activity of CPW and MBL between hens with “early life and adult heat experience” and hens with “adult heat experience” only (Table 2).

A difference was observed in complement activity of APW ($P < 0.05$; Table 2). This difference was, however, not caused by the actual heat stress. The heat stress treatments differed in APW activity before the start of heat stress at 24 wk of age.

A difference was also observed in corticosterone levels ($P < 0.05$; Table 2), in which corticosterone levels of hens with “adult heat experience” were higher than corticosterone levels of hens with “early life and adult heat experience.” This difference was probably not caused by the actual heat stress. Before the start of heat stress, corticosterone levels of hens with “adult heat experi-

ence” were already higher than corticosterone levels of hens with “early life and adult heat experience.”

LPS Experience

One laying hen with early life LPS experience died within 1 d after repeated LPS administration at 24 wk of age. Data of this bird were not included in the statistical analysis.

Levels of Ab binding to LPS were increased after LPS administration. Hens with “early life and adult LPS experience” had a different response level and response pattern compared with hens with “adult LPS experience” (treatment × time interaction, $P < 0.0001$; Table 3). Hens with “adult LPS experience” showed a primary immune response with a peak in Ab level at d 7 after LPS administration. Hens with “early life and adult LPS experience” showed a secondary immune response with a peak in Ab level at d 4 after LPS administration (Figure 1).

Levels of natural Ab binding to KLH were increased after LPS administration. Hens with “early life and adult LPS experience” had a different response pattern compared with hens with “adult LPS experience”

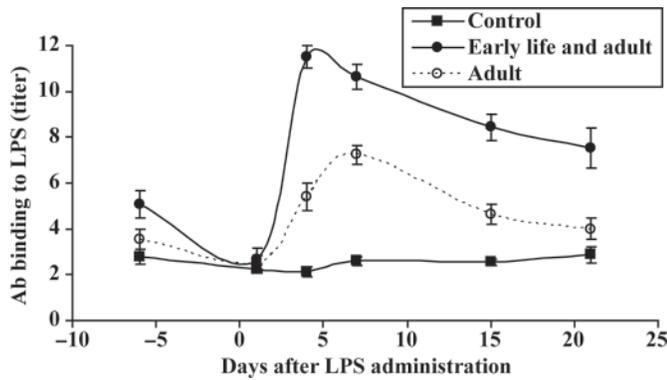


Figure 1. Effect of early life exposure to *Escherichia coli* lipopolysaccharide administration (LPS; i.v., 1 mg/kg of BW) on level of antibody (Ab) binding to LPS in later life. Treatment “early life and adult” were laying hens administered with LPS at 6 wk of age and repeated administration of LPS at 24 wk of age, whereas treatment “adult” were laying hens only administered with LPS at 24 wk of age.

(treatment \times time interaction, $P < 0.01$; Table 3). Hens with “adult LPS experience” had a peak in natural Ab level at d 7 after LPS administration, whereas hens with “early life and adult LPS experience” had a peak

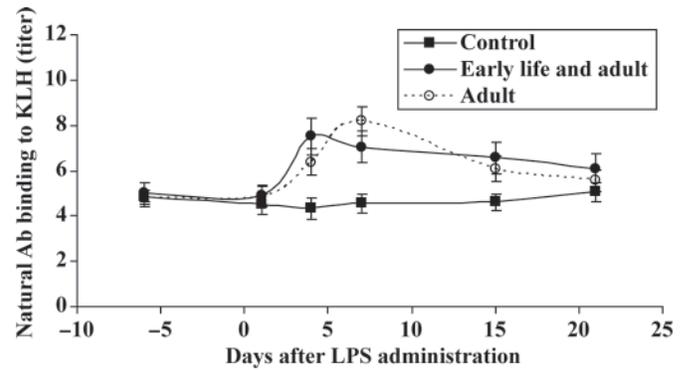


Figure 2. Effect of early life exposure to *Escherichia coli* lipopolysaccharide administration (LPS; i.v., 1 mg/kg of BW) on level of natural antibody (Ab) binding to keyhole limpet hemocyanin (KLH) in later life. Treatment “early life and adult” were laying hens administered with LPS at 6 wk of age and repeated administration of LPS at 24 wk of age, whereas treatment “adult” were laying hens only administered with LPS at 24 wk of age.

in natural Ab level at d 4 after LPS administration (Figure 2).

No differences were found for BW development in time; hen-day egg production; complement activity of

Table 3. Effect of early life exposure to *Escherichia coli* lipopolysaccharide (LPS) administration (i.v., 1 mg/kg of BW) on performance, immune, and endocrine parameters in later life¹

Parameter		LPS treatment			P-value ²		
		Control	Early life and adult	Adult	Treatment	Time	Treatment \times time
Number of birds		12	10	12			
Feed intake ³ (g/d)	P1	79.0	91.1	81.6	0.2411		
	P2	95.0	77.3	70.7	0.4648		
	P3	103.5	100.9	97.5	0.6825		
Egg production ³ (%)	P1	59.0	70.5	50.9	0.2711		
	P2	89.3	34.3	27.0	0.5223		
	P3	90.0	90.7	77.8	0.2481		
BW gain ⁴ (g)		-9	14	27	0.6517	0.0042	0.9389
Egg weight (g)		52.7	56.1	53.5	0.0482	<0.0001	0.3746
Complement (CH50 ⁶ -value) (μ g/mL)	CPW ⁵	522	492	538	0.3414	<0.0001	0.6665
	APW ⁷	210	192	220	0.1511	<0.0001	0.7842
	MBL ⁸	14.0	17.8	19.0	0.5937	<0.0001	0.2993
Antibody titer	LPS ⁹	2.6	7.1	4.6	0.0034	<0.0001	<0.0001
	KLH ¹⁰	4.7	5.8	6.0	0.7375	<0.0001	0.0012
Corticosterone (ng/mL)		2.7	2.2	2.1	0.6606	0.0028	0.2132

¹Treatment “early life and adult” are laying hens administered with LPS at 6 wk of age and repeated administration of LPS at 24 wk of age, whereas treatment “adult” are laying hens only administered with LPS at 24 wk of age.

²Differences between “early life and adult experience” and “adult experience.” Data of the control group were not statistically compared due to the interferences between early life and adult experience.

³Data on feed intake and hen-day egg production were divided into 3 periods: P1 is the adaptation period (d -11 to 0), P2 is the first week of the experimental period (d 1 to 7), and P3 is the second and third week of the experimental period (d 8 to 22).

⁴The BW development in time was calculated compared with BW at d 5 before the start of heat stress.

⁵CPW = classical complement pathway.

⁶CH50 = titer that lyses 50% of the red blood cells.

⁷APW = alternative complement pathway.

⁸MBL = mannan-binding lectin.

⁹LPS = lipopolysaccharide.

¹⁰KLH = keyhole limpet hemocyanin.

CPW, APW, and MBL; and corticosterone level between hens with “early life and adult LPS experience” and hens with “adult LPS experience” (Table 3).

DISCUSSION

In the present study, effects of early life experience with climatic (heat) or hygienic (LPS) stressors on performance, humoral immune competence, and endocrine responsiveness after reexposure to the same stressor in later life were investigated in laying hens. The focus was on the responses in later life, rather than the responses of the chicks exposed to the early life stressors. The purebred layer line used to test the hypothesis has been used in previous studies (Star et al., 2007, 2008a,b), in which adult hens were exposed to a single or combined climatic stress (heat) and hygienic stress (LPS) during early lay. Hens of this line were not able to maintain a high hen-day egg production during heat stress and showed more fluctuations in humoral immune responsiveness compared with 3 other purebred layer lines, which might indicate that the adaptability of this line to the stressors was suboptimal and therefore a good candidate to study the possibility to improve the adaptability of laying hens.

All performance, immune, and endocrine parameters were only measured in adult laying hens at similar time points as in previous studies (Star et al., 2007, 2008a,b). The aim of the present study was to improve adaptability to heat stress by early life exposure, in which adaptability can be defined as the ability of an individual to maintain feed intake, production, behavior, and other features of life in response to environmental changes. In broilers, several studies (Arjona et al., 1990; Yahav and Hurwitz, 1996; Yahav and Plavnik, 1999; De Basilio et al., 2001; Yahav and McMurtry, 2001) showed an improved adaptability to heat stress in later life (i.e., improved performance at marketing age, based on growth, feed intake, and feed efficiency between the period of early life and later life heat exposure). In the present study, however, early life exposure to heat stress did not improve adaptability of laying hens during reexposure to heat stress in later life, based on these performance parameters. There are several explanations why early life heat exposure in layers did not result in the expected improvement of adaptability: 1) laying hens may have a sufficient thermoregulatory system irrespective of early life heat exposure; 2) difference in growth rate and, related to this, in heat production between broilers and layers; 3) time between first and second heat exposure was about 23 wk, which is a long period compared with the 4 to 5 wk between the first and second heat exposure in broilers; 4) during reexposure to heat stress, temperature was lower (32°C) and time of exposure was longer (22 d) compared with studies done in broilers [temperature (above) 35°C, 6 to 8 h]; and 5) the period of early life heat exposure was based on a physiological sensitive period for imprinting;

however, this might not be the right period in which to manipulate the thermoregulatory system of layers.

Responses to LPS administration within the present and previous studies were comparable for performance parameters; feed intake, BW, hen-day egg production, and egg weight were decreased in the first week after LPS administration. Although early life LPS exposure did not improve performance of laying hens reexposed to LPS in later life, early life LPS exposure significantly influenced the kinetics and magnitude of the response of Ab binding to LPS in later life. Hens administered with LPS only at adult age showed an immune response with primary characteristics with a peak in Ab level at d 7 after LPS administration, whereas hens with early life and adult LPS experience had an accelerated and higher peak in Ab level at d 4 after LPS administration and showed a secondary immune response. The observed change in the kinetics and magnitude by early life LPS exposure indicated a classic T-cell-dependent Ab response (memory) to LPS, as also observed after intratracheal administered LPS in laying hens (Ploegaert et al., 2007). This is, however, in contrast with the established concept that Ab responses to LPS are T-cell-independent [as mentioned for instance by Gehad et al. (2002)] and, therefore, no memory to LPS should be developed.

In the previous studies, it has been shown that levels of natural Ab binding to KLH increased after LPS administration, and the present study showed that early life LPS exposure influenced the kinetics of the response of natural Ab binding to KLH. Hens with early life LPS experience had a peak in natural Ab level to KLH at d 4 after LPS administration and hens administered with LPS only at adult age had a peak in natural Ab level to KLH at d 7 after LPS administration. It is not clear yet why natural Ab binding to KLH is enhanced after LPS challenge. It seems, however, that the response is B-cell-related and that change in kinetics is based on B-cell memory.

A 2.5-fold increase in activity of MBL was induced 1 d after LPS administration (Figure 3). Other studies (Laursen and Nielsen, 2000; Juul-Madsen et al., 2003) also found an upregulation of MBL activity in chickens during the acute stages of infections. These studies confirm that MBL binds to LPS and initiates a proinflammatory response (Kang et al., 2007). In the present study, however, there was no difference in the upregulation of MBL activity between hens with early life LPS experience and hens only administered with LPS as adults. This indeed suggests that binding of LPS by MBL initiates a proinflammatory response irrespective of any experiences with LPS, indicating the short-term severity of this endotoxin. In addition, the short-term severity of LPS might be the reason why no effect was observed on performance parameters and hens did not become refractory to repeated LPS administrations, which is in contrast to the findings by Korver et al. (1998) and Parmentier et al. (2006).

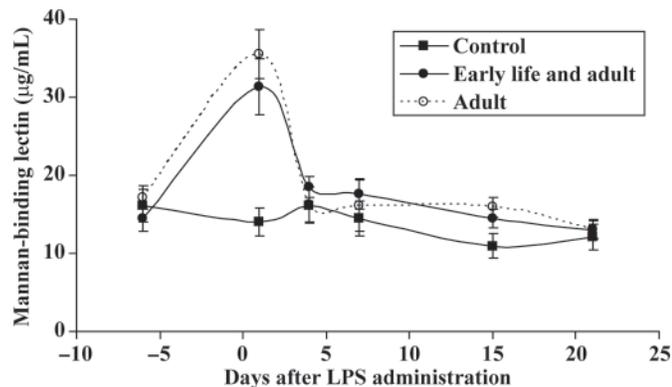


Figure 3. Effect of early life exposure to *Escherichia coli* lipopolysaccharide administration (LPS; i.v., 1 mg/kg of BW) on activity of the mannan-binding lectin pathway of complement in later life. Treatment “early life and adult” were laying hens administered with LPS at 6 wk of age and repeated administration of LPS at 24 wk of age, whereas treatment “adult” were laying hens only administered with LPS at 24 wk of age.

As for heat treatment and LPS administration, both “early life and adult experience” and “adult experience” groups differed from the control group. For LPS administration, the control group served as a real control because the hens were housed in the same chamber as the LPS-administered groups during the experimental period. The control group was housed in a different chamber as the heat-treated groups, so whether or not the control group served as a real control can be argued. However, the difference between the heat-treated groups and the control group gives a good impression on the effect that heat stress can have on adult laying hens. Furthermore, the birds were housed from climate chambers (first week after hatch), to a rearing facility, to climate chambers again. Because all birds were moved and housed all together each time (except for the heat-treated chicks at d 5 of age), this probably did not influence the results of this experiment. The results of heat and LPS treatment in the groups “adult experience” and the control group were also comparable to the results of previous studies (Star et al., 2007, 2008a,b), indicating that moving and housing of the birds did not influence the results. Therefore, it can be concluded that the present data revealed no positive effect of early life heat stress exposure on the adaptability of laying hens in later life. Furthermore, early life LPS experience did not affect adaptability in terms of performance and endocrine responsiveness. Early life LPS experience, however, affected kinetics and magnitude of (natural) Ab levels binding to LPS and to KLH, indicating that early life LPS experience can enhance the status of immune reactivity or induce a higher sensitivity to LPS.

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