Susceptibility to pre-natal stress in laying hens: Effects of genetic strain and maternal age on growth rate of the offspring

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Summary

Maternal stress during egg formation influences the development of offspring through direct and epigenetic mechanisms. The aim of this study was to investigate thematernal age offspring susceptibility to pre-natal stress in laying hens. This was tested by subjecting breeder hens of 5 genetic lines — ISA Brown, Lohmann Brown, LSL-Lite, Shaver White and Pure Line White Leghorn — to natural stressors and by pharmacological *in ovo* injections of corticosterone prior to incubation. Eggs of four parent flocks (24F:3M/flock) from each genetic line were incubated, hatched, reared, and housed identically in litter floor pens with nests and perches. Parent stock was equally separated into two groups: Stress, where hens were subjected to a series of acute psychological stressors (e.g. physical restraint) for 8 days prior to egg collection, and Control, which received routine husbandry. At 32 and 52 weeks of age (woa), fertile eggs from both treatments were collected and additional eggs from Control group were injected with corticosterone (10ng/mL eggCORT). A vehicle treatment (CORT Control) was included to for effects of egg manipulation. redistributedbirds Offspring growth was affected by the interaction of genetic line and treatment for both natural (P=0.0091) and pharmacological (P=0.0063) stressors. Average body weight decreased in response to natural stressors only in ISA Brown (P=0.0346). and PureLine White Leghorn offspring were neither affected by stressor (P=0.4150) nor maternal age (P≥0.0712). These results show that pre-natal stress, genetic line, and maternal age interact to affect growth rate of the offspring in commercial strains of laying hens.

Keywords: laying hens, genetics, breeder flock, prenatal stress, corticosterone

Introduction

The process of selection during domestication in chickens from their wild ancestor, the Red Junglefowl, resulted in several differences in morphology, physiology and behaviour, such as faster growth and reduced fearfulness in the domesticated chicken (Campler et al., 2009; Price, 2009). The development of the domestic hen is largely determined by the combination of genotype, environment and early-life experience. Additionally, environmental stressors experienced by the mother can modify gene expression patterns and affect stress susceptibility resulting in phenotypic impairments of the offspring (Henriksen et al., 2011). It has been

suggested that domesticated chickens are more prone to transgenerational effects of maternal stress than their ancestors (Nätt et al., 2012). Therefore, genetic differences between traditional pure lines and highly selected commercial hybrids might also influence transgenerational susceptibility to stress.

In avian species, like in mammals, glucocorticoids are produced by the adrenal glands in response to stressors (Henriksen et al., 2011). Effects of stress can be modelled by imposing natural stressors on parents or *in ovo* injections of corticosterone, the main glucocorticoid in birds. If it is found that changes in stress response due to experience of the breeder flock are readily transferred to the offspring, it could improve selection for resilient traits and help to explain why significant flock to flock variation in birds of the same strain receiving similar husbandry.

Material and methods

Parent stock and housing

Fertilized eggs of parent stock were provided by Hendrix Genetics (ISA Brown and Shaver White), Lohmann Tierzucht (Lohmann Brown and LSL-Lite), and the University of Guelph's Arkell Research Station (Pure Line White Leghorn). All grandparent hens from which eggs were collected were between 40 to 50weeksold. Eggs were stored at 4ºC until incubation and hatched at the hatching facility of the University of Guelph's Arkell Research Station. were sexed, identified and placed into floor pens where they were raised under the same conditions. At 16 woa, each strain was equally distributed to 4 parent flocks of 27 birds (24 females:3 males) and assigned either to Control or Stress treatments (2 reps/treatment/strain).

Treatments and egg collection

Control Stress
At 31 and 51 woa, hens from Stress group were submitted to 8 consecutive daily sessions of stressors: on days 1 and 5, hens were transferred to crates and transported outside the research facility for 15 minutes; on days 2 and 6, birds were individually physically restrained in a cloth bag for 10 minutes; on days 3 and 7, birds were crated, transferred to a novel environment and submitted to 3 simulated aerial predator attacks using a cardboard sparrowhawk; on days 4 and 8, birds were placed in crates outside the barn and submitted to auditory stressor using an air horn. The stressors were applied at random times between 9:00 and 16:00h. Control groups were not exposed to any experimental stress at any stage. Eggs laid on days 8 and 9 after the beginning of were collected from the Stress groups. Egg collection from groups started 2 days before the beginning of and lasted 11 days. All eggs were stored at 4ºC until incubation.

CORT CORT Control

A sample of eggs from the Control group received injections of 0.6g of corticosterone (Sigma) diluted in 60l sesame oil (Fisher) prior incubationconcentration was determined based on egg weight, assuming 10% shell and 90% egg content (Beuving et al., 1981). On average, eggs consisted of 60g liquid so that CORT injections achieved egg concentrations of 10 ng/ml. A

vehicle treatment (CORT Control) in which eggs received an injection with 60l of sesame oil alone, was included to control for effects of egg manipulation.

Offspring incubation, housing and body weight

Eggs from all treatments were incubated and hatched under identical conditions. Males and females of each strain and treatment were individually identified and equally distributed to pens measuring 3.72m^2 with perches and litter floor. Control and Stress treatments had 2 of 14 chicks per strain, whereas CORT and CORT Control had 2 of 10 birds per strain due to lower hatchability. All birds were individually weighed at 0 (hatch), 2, 4, 8, 13, 15 and 17 woa. Biweekly body weights (g) were recorded and analysed over time.

Statistical methods

The Glimmix procedure of SAS Ver. 9.4 (SAS Institute, Cary, NC) was used to perform all statistical analysesseparate analyses were ne for differences between natural Stress versus Controlhe second for differences controland . A generalized linear mixed model was used to analyze the data for body weight from 0 to 17 woa. Both models included fixed effects of: treatment, maternal age, genetic line, sex and and random effects of room and pen. genetic linegenetic lineBody weights were log-normal transformed to meet the assumption of a normal distribution of residuals. The Least Square means and standard errors were back-transformedand are presented in the results for all response variables. The random effect was partitioned with pen nested within room and the model accounted for unbalanced repeated measures.

Results and Discussion

Average body weight of the offspring from 0 to 17 woa was affected by the interaction of genetic line and natural stressor (P=0.0091) (Table 1). Stress offspring was lighter than Control (P=0.0346).

1. Average body weight (mean ± S.E) strain.

Genetic Line	Control	Stress	Control	CORT Control	CORT
ISA B.	**524.9±3.0^a**	510.77±3.1^b	**524.9±3.0^a**	516.02±3.8^{ab}	496.60±4.7^b
L. Brown	**525.4±3.0**	524.56±2.8	**525.4±3.0^a**	499.53±4.4^b	471.02±6.8^c
S. White	**472.3±2.0**	475.85±2.1	**472.3±2.0^a**	459.96±2.6^b	441.21±3.3^c
LSL-Lite	**473.7±1.7**	466.07±2.0	**473.7±1.7^a**	466.58±2.5^{ab}	451.05±4.8^b
W.Leghorn	**440.3±2.8**	443.71±3.2	**440.30±2.8**	426.90±4.1	425.96±5.6

0.05).

For pharmacological , the average body weightfrom 0 to 17 woa for both maternal ages combined was also affected by the interaction of genetic line and (P=0.0063) (Table 1). CORT consistently decreased growth rate in all 4 commercial lines (P≤0.0022), but no differences were seen Pure Line White Leghorn (P=0.4150).

There was a treatment by strain by maternal age interaction (P<0.0001). stress decreased average body weight in brown lines at maternal age of 32 woa (P≤0.0014) white commercial lines from 52 woa mothers were affected (P<0.0001). Line White Leghorn was not affected by maternal

age (P≥0.0712) (Table 2).

2. Average body weight (mean ± S.E.) at 32 and 52 weeks of maternal age.

	32			52		
GeneticLine	Control	CORT Control	CORT	Control	CORT Control	CORT
ISA Brown	**533.4±3.^{a}**	**508.3±6.2^{ab}**	499.±6.3^{c}	**516.6±4.9**	523.8±4.5^{ab}	493.8±6.9^{b}
L. Brown	**524.7±4.2^{a}**	**487.3±7.7^{b}**	459.5±10.7^{b}	**526.1±4.1^{a}**	51.±3.^{ab}	482.±8.3^{b}
S White	**462.4±2.5**	**455.4±4.7**	443.±4.	**482.4±2.1^{a}**	464.5±2.3^{ab}	439.±4.^{b}
LSL-Lite	**471.±2.5**	**453.±3.0**	457.±8.	**476.0±3.2^{a}**	480.±4.0^{a}	445.1±4.4^{b}
Leghorn	**433.8±3.6**	**420.3±6.1**	434.±8.2	**450.±3.8**	433.±5.3	417.5±7.8

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Conclusion

These results show that response to pre-natal stress in commercial laying hens can be affected by both genetic line maternal age. he natural stress model may be useful for quantifying the response , whereas the pharmacological model may be useful for quantifying the response of the embryo.

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