

RESEARCH ARTICLE

Growth and innate immunity are not limited by selection for high egg testosterone content in Japanese quail

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SUMMARY

The effects of maternal androgens on fitness-related traits of offspring are generally assumed to be epigenetic adaptations to the environment that may be encountered by the next generation. Possible constraints of high yolk androgen transfer are still not understood, although a suppressed immune response in offspring is frequently considered. The aim of our study was to examine the innate immune defence in high (HET) and low egg testosterone (LET) lines of Japanese quail, which differ in the hormonal milieu of their eggs, thus providing a good physiological model for the study of androgen-mediated maternal effects. Acute phase response was induced by a lipopolysaccharide injection in 12-day-old quail and plasma corticosterone and the heterophil:lymphocyte ratio were measured at 1 and 3 h post-treatment. Basal levels of non-specific antibodies (IgY) were determined in the circulation. We found that HET quail were heavier than LET quail from the second week of age, indicating enhanced post-hatching growth. At 1 h post-lipopolysaccharide challenge, plasma corticosterone concentrations increased in the HET but not in the LET line. The heterophil:lymphocyte ratio rose in both lines at 3 h post-immune challenge, with a more pronounced response in HET quail. Moreover, HET chicks displayed higher IgY levels than LET chicks, suggesting either enhanced passive immunoprotection or stimulated endogenous antibody production. In conclusion, our data demonstrate that the genetic selection for high egg testosterone content positively influences growth and, simultaneously, does not limit the acute phase response in young quail.

Key words: yolk androgens, growth, innate immunity, corticosterone, plasma antibodies.

INTRODUCTION

The transgenerational transfer of maternal hormones to a developing embryo can mediate epigenetic adaptations to the environmental conditions that may be encountered by the next generation (Mousseau and Fox, 1998). Previous studies have demonstrated that yolk androgens can exert pleiotropic effects on the offspring's phenotype, with consequences that extend into adulthood (Groothuis et al., 2005b; Gil, 2008). For example, experimental manipulations of androgen content in the yolk, especially testosterone (T) and androstenedione, have been found to influence postnatal growth (Schwabl, 1996), immunocompetence (Müller et al., 2005; Tobler et al., 2010) as well as competitive and agonistic behaviour within both non-reproductive (Eising and Groothuis, 2003; Müller et al., 2009) and sexual contexts (Eising et al., 2006; Partecke and Schwabl, 2008).

To explain these diverse effects, yolk androgens could either independently organize the different pathways responsible for different body functions or specifically target only one system, resulting in indirect consequences on others (Groothuis and Schwabl, 2008). For example, a redistribution of energetic demands into one system at the expense of other functions can account for the balance between beneficial and detrimental effects of elevated yolk androgens found in offspring. Such negative correlations have been demonstrated for growth rate and the activity of the immune system (Groothuis et al., 2005a; Navara et al., 2005); however, several other studies have failed to provide evidence to support this relationship

(Müller et al., 2005; Tschirren et al., 2005; Pitala et al., 2009). Therefore, it seems that yolk androgens can influence immunity and growth independently of each other. An exposure to increased yolk androgens depressed both the humoral and cell-mediated immune response in black-headed gull (*Larus ridibundus*) during the early chick phase (Müller et al., 2005) and in jackdaw (*Corvus monedula*) later during the nestling period (Sandell et al., 2009). In contrast, experimentally increased yolk T content stimulated cell-mediated immunity in house finch (*Carpodacus mexicanus*) nestlings (Navara et al., 2006) and the humoral immune response in zebra finch (*Taeniopygia guttata*) adults (Tobler et al., 2010). Thus, the current findings point out the immunomodulatory effects of maternal androgens varying in the type of immune response, the developmental stage and within the context of the prevailing environmental conditions (Tobler et al., 2010). Furthermore, a dose-dependent experiment in grey partridge (*Perdix perdix*) demonstrated an enhanced and decreased immune response of chicks hatched from eggs treated with low and high T doses, respectively (Cucco et al., 2008). Such a U-shaped dose–response curve clearly points out the limitations of yolk hormone manipulation by a single injection into the egg and suggests that not only dose but also inter-female differences in endogenous yolk hormone concentrations should be considered before experimental manipulation.

As most experimental studies have investigated the effects of yolk androgens on cell-mediated and humoral immunity, we focused on the acute phase response (APR) as an early line of non-specific (innate)

immune defence triggered by injury or various infection attacks. The APR represents a systemic immune reaction with characteristic behavioural, metabolic and endocrine changes that contribute to a general assessment of immunocompetence (Baumann and Gauldie, 1994; Owen-Ashley and Wingfield, 2007). The aim of the present study was to analyse immune activation during the APR induced by lipopolysaccharide (LPS) injection in two lines of Japanese quail (*Coturnix japonica*) divergently selected for high (HET line) and low (LET line) egg T content. The APR was examined by plasma corticosterone concentrations and the heterophil:lymphocyte (He:Ly) ratio in 12-day-old chicks. In addition, we measured basal levels of non-specific antibodies (IgY) in the circulation of chicks. These immunoglobulins may be of both maternal and endogenous origin because maternal antibodies pass through the yolk into the embryo (Brambell, 1970) and endogenous IgY-bearing cells have been detected during the embryonic development (Kincade and Cooper, 1971), although endogenous synthesis of antibodies begins to dominate after 2 weeks of age (Watanabe and Nagayama, 1979; Grindstaff, 2008). Artificial selection for yolk T concentrations has proved a heritable variation of yolk hormone transfer accounting for nearly half of its total phenotypic variance (Okuliarova et al., 2011b). Thus, the evolutionary response of yolk hormone deposition may be affected by the selection pressure on yolk hormone-mediated traits in offspring. Because HET quail, which are exposed to high T concentrations in the egg, displayed enhanced growth in comparison with LET quail (Okuliarova et al., 2011a), we predicted weaker immune response in the HET line than the LET line as a possible constraint of yolk T deposition.

MATERIALS AND METHODS

Animals and breeding conditions

Two lines of Japanese quail, *Coturnix japonica* Temminck and Schlegel 1849, divergently selected for yolk T concentrations were used in the experiment. Animals for the parental stock were obtained from the third generation of the HET and LET lines bred at the Institute of Animal Biochemistry and Genetics, Slovak Academy of Sciences (IABG), Ivanka pri Dunaji, Slovak Republic (Okuliarova et al., 2011b). We used 27 females and 15 males from the LET line and 25 females and 13 males from the HET line. Birds at the age of 21 weeks were housed two females with one male per cage (except for four cages containing one female with one male) under a stimulatory photoperiod (14 h:10 h light:dark). Food and water were provided *ad libitum*. The mean (\pm s.e.m.) yolk T levels were 6.8 ± 0.3 pg mg⁻¹ yolk and 15.6 ± 0.9 pg mg⁻¹ yolk in the LET and HET lines, respectively.

On average, three to four eggs per female were collected (98 LET and 83 HET). The egg mass did not differ between the HET and LET lines (mean \pm s.e.m.: LET, 9.55 ± 0.07 g; HET, 9.53 ± 0.07 g; $t=0.149$, d.f.=179, $P=0.882$). Eggs were then incubated in a forced draught incubator (BIOSKA, Sedlčany, Czech Republic) at a temperature of $37.5 \pm 0.2^\circ\text{C}$ and relative humidity of 50–60%. Hatching success was comparable in both lines (LET 42.9% and HET 47.0%; $\chi^2=0.17$, $P=0.684$). As all eggs (per each line) were incubated together, we were unable to control mother identity of hatchlings in our experimental design. After hatching, 32 chicks from each line were marked by colour rings on their legs and assigned to four boxes according to the line (two LET and two HET). They were reared under constant light with food (a starter feeding mash for young turkeys) and water provided *ad libitum*. Temperature was decreased stepwise from 37–35°C after hatching to 27–28°C at the end of the experiment. Body mass was recorded at 1, 4, 8 and 11 days post-hatching.

The experiment was approved by the Ethical Committee of the IABG (licence number SK PC 22004).

LPS challenge

The APR was induced by intraperitoneal injection of LPS from the cell wall of *Escherichia coli* (Sigma-Aldrich, St Louis, MO, USA, cat. no. L3012). At the age of 12 days, the quail chicks were administered either with 1.5 mg LPS per 1 kg of body mass dissolved in 100 μ l phosphate buffer saline (PBS) (LET: $N=15$; HET: $N=14$) or with 100 μ l PBS (LET: $N=16$; HET: $N=15$). Such LPS doses have been shown to prevent the development of sickness symptoms but be sufficient to stimulate immune and stress responses (Koutsos and Klasing, 2001).

One half of the chicks were then decapitated 1 h, and the next half 3 h, after LPS treatment. Blood was collected into heparinised tubes and used for white blood cell (WBC) differential counts and corticosterone and IgY assays. Moreover, the bursa of Fabricius was dissected and weighed. The sex of the animals was determined by the visual inspection of the gonads.

WBC differential counts

WBC counts were analysed on blood smears that were prepared from 5 μ l of the whole blood. Dried blood smears were fixed and stained as follows: 3 min May–Grünwald solution, 1 min May–Grünwald solution with neutral water (1:1) and 20 min Giemsa–Romanowsky solution with neutral water (1:10). Relative counts of He, Ly, eosinophils, monocytes and basophils were examined per 200 leukocytes on each slide at 1000 \times magnification using an immersion light microscope (CARL ZEISS, Jena, Germany). Basophils were not further analysed because of their minor frequencies in the blood.

Corticosterone assay

Plasma corticosterone concentrations were measured by radioimmunoassay using a Corticosterone Rat/Mouse ¹²⁵I-labelled RIA kit (DRG Instruments GmbH, Marburg, Germany, cat. no. 1364) that was validated for the measurement of quail plasma samples. All samples were run within two assays.

Antibody assay

Total antibody levels in the quail plasma were determined by enzyme-linked immunosorbent assay (ELISA) following the protocol set out by Grindstaff et al. (Grindstaff et al., 2005). Plates were coated with anti-chicken IgY (Sigma-Aldrich, cat. no. C6409) diluted 1:800 in carbonate buffer (pH 9.6) and incubated overnight at 4°C. After a wash (3 \times 100 μ l PBS–0.05% Tween 20), plates were blocked with 2.5% milk powder (diluted in PBS) and incubated for 2 h at 37°C. Plasma samples were diluted 1:25,000 in 0.2% milk powder in PBS. Following a wash, the diluted samples were added to the plates and incubated overnight at 4°C. All samples were measured in duplicate within two plates, and chicken IgY standard (Promega, Madison, WI, USA, cat. no. G1161) serially diluted from 6.25 ng ml⁻¹ to 400 ng ml⁻¹ was included on each plate. Following a wash, alkaline-phosphatase-conjugated anti-chicken IgY (Sigma-Aldrich, cat. no. A9171) diluted 1:1000 in 0.2% milk powder in PBS was added to the plates and incubated for 1 h at 37°C. Plates were washed and a substrate buffer with p-nitrophenyl phosphate (Merck, Darmstadt, Germany) was added to each well and incubated at room temperature in the dark. After 25 min incubation, the reaction was stopped by 1 mol l⁻¹ sodium hydroxide. Absorbance was measured at a wavelength of 405 nm in an ELISA reader (EL800 Bio-Tek Instruments, Winooski, VT, USA).

Statistical analysis

All data were subjected to a Kolmogorov–Smirnov test to determine whether they fit a normal distribution. If not, an appropriate transformation was applied. Body mass from hatching until 11 days of age was analysed by repeated-measures ANOVA, including line and sex as fixed factors, age as the repeated factor and all interactions. The mass of the bursa of Fabricius corrected for body mass was log-transformed and analysed by two-way ANOVA (line and sex as fixed factors). For the proportions (percentages of monocytes and eosinophils), arcsine square-root transformation was applied. Data for the He:Ly ratio and corticosterone were log-transformed. Differences in WBC counts, the He:Ly ratio and plasma corticosterone concentrations were examined with a three-way ANOVA, in which the effects of line, LPS treatment and time post-treatment were tested. As these variables were not influenced by the sex of the offspring, this effect was excluded from the analysis. If the interactions were significant, differences between individual groups were evaluated by Fisher's least significant difference (LSD) *post hoc* tests. Body mass was included as a covariate in the analysis of immune measures (He:Ly ratio, corticosterone and plasma IgY) and because of a non-significant effect, it was removed from the final statistical model. Independent *t*-tests were used to compare the plasma IgY levels and egg mass of the two lines. Hatchability was calculated from all laid eggs and compared using a chi-square test.

RESULTS

Early growth and immune organ mass

Statistical analysis of body mass revealed significant main effects of line ($F_{1,56}=5.34$, $P<0.05$), sex ($F_{1,56}=6.72$, $P<0.05$) and age ($F_{3,168}=4620$, $P<0.001$), as well as significant interactions of age \times line ($F_{3,168}=10.31$, $P<0.001$) and age \times sex ($F_{3,168}=6.04$, $P<0.01$). No line differences in body mass were found in the hatchlings ($P=0.867$), but HET quail were heavier than LET quail at 8 ($P<0.05$) and 11 days of age ($P<0.001$; Fig. 1). Similarly, body mass differences between sexes arose at 8 ($P<0.05$) and 11 days of age ($P<0.01$), when females became heavier than the males.

The mean mass of the bursa of Fabricius, corrected for body mass (mg g^{-1}), did not differ between the LET (males: 0.815 ± 0.055 ; females: 0.901 ± 0.075) and HET lines (males: 0.734 ± 0.056 ; females: 0.990 ± 0.070) in 12-day-old quail ($F_{1,56}=0$, $P=1.00$). However, the relative bursa mass was higher in females compared with males in both lines ($F_{1,56}=6.91$, $P<0.05$).

LPS challenge and plasma IgY levels in chicks

LPS administration increased plasma corticosterone concentrations at 1 h post-injection only in the HET line, without any effects in the LET line (Table 1, Fig. 2A). Thus, the LPS-injected HET group displayed higher plasma corticosterone levels compared with the

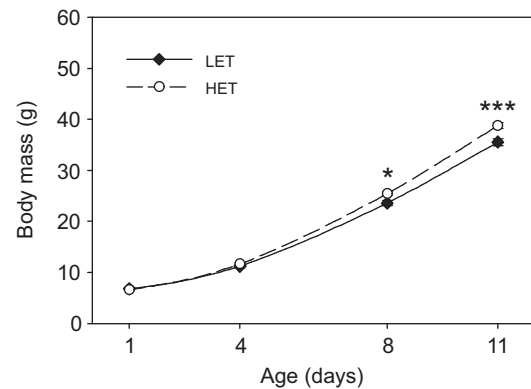


Fig. 1. Early body mass (mean \pm s.e.m.) of Japanese quail selected for high (HET) and low egg testosterone (LET) content. * $P<0.05$; *** $P<0.001$.

PBS-injected HET group ($P<0.01$) and both the LPS- and PBS-injected LET groups ($P<0.01$ for both). No responses to the LPS, as well as no line differences in plasma corticosterone, were found at 3 h post-treatment.

Mean WBC differential counts are given in Table 2. LPS administration significantly affected leukocyte counts 3 h after treatment, without any changes at 1 h post-LPS injection. LPS increased He and decreased Ly counts, with a subsequent increase in the He:Ly ratios in both the LET ($P<0.001$) and HET ($P<0.001$) lines (Table 2). HET quail displayed a significantly higher He:Ly ratio in response to LPS treatment compared with LET quail ($P<0.01$), whereas no line differences were recorded in groups after PBS injection (Fig. 2B). The relative percentage of monocytes decreased after LPS administration in the HET line ($P<0.05$), but was not affected in the LET line (Table 1). The relative percentage of eosinophils did not alter in response to the LPS challenge (Table 1).

The lines were compared for circulating basal IgY levels by grouping both PBS-injected groups (1 and 3 h post-injection). HET quail exhibited significantly higher plasma antibody levels compared with LET quail ($t=3.694$, d.f.=27, $P<0.001$; Fig. 3).

DISCUSSION

We analysed a non-specific immune defence activated during the APR in Japanese quail chicks from two lines divergently selected for yolk T content. The embryonic development of the HET and LET lines significantly differs by the hormonal milieu in the egg, providing a physiological model for the study of hormone-mediated maternal effects (Okuliarova et al., 2011b).

In response to the LPS challenge, plasma corticosterone concentrations increased in the HET quail whereas no such response was found in the LET quail at 1 h post-LPS injection. An activation

Table 1. Three-way ANOVA for plasma corticosterone (CORT) concentrations, white blood cell differential counts and the heterophil:lymphocyte (He:Ly) ratio in Japanese quail, reporting the effects of line, LPS treatment, time post-treatment and all interactions

Factor	Plasma CORT		Heterophils		Lymphocytes		Eosinophils		Monocytes		He:Ly ratio	
	$F_{1,51}$	P	$F_{1,52}$	P	$F_{1,52}$	P	$F_{1,52}$	P	$F_{1,52}$	P	$F_{1,52}$	P
Line	3.71	0.059	0.03	0.872	0.05	0.829	0	0.992	0.23	0.630	0.69	0.411
LPS treatment	5.25	<0.050	34.85	<0.001	35.76	<0.001	0.03	0.867	1.03	0.253	33.29	<0.001
Time	0.35	0.558	23.87	<0.001	24.91	<0.001	0.07	0.797	0.07	0.703	24.85	<0.001
Line \times LPS treatment	7.83	<0.010	0.30	0.584	0.16	0.694	0.11	0.740	0.11	<0.050	1.16	0.287
Line \times Time	0	0.977	0.44	0.510	0.21	0.647	1.03	0.315	1.03	0.194	1.70	0.198
LPS treatment \times Time	5.02	<0.050	15.10	<0.001	15.63	<0.001	0.03	0.858	0.03	0.930	18.92	<0.001
Line \times LPS treatment \times Time	11.78	<0.010	3.82	0.056	3.54	0.067	2.01	0.162	2.01	0.640	4.10	<0.050

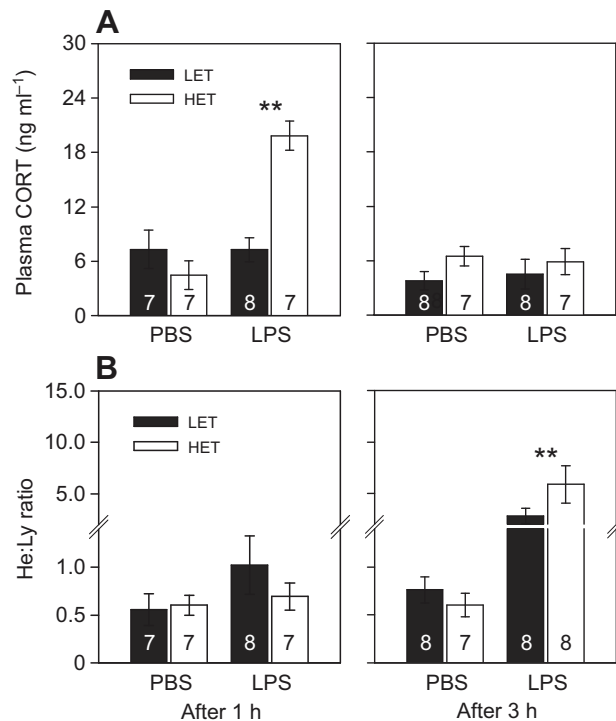


Fig. 2. Plasma corticosterone (CORT) concentrations (A) and the heterophil:lymphocyte (He:Ly) ratio (B) at 1 and 3 h post-lipopolysaccharide (LPS) challenge in 12-day-old Japanese quail selected for high (HET) and low egg testosterone (LET) content. Data are means \pm s.e.m. Only significance levels for line differences are plotted. ** $P<0.01$.

of the hypothalamic–pituitary–adrenal (HPA) axis followed by a corticosterone release is one of the successively triggered steps involved in the APR that can be experimentally induced by bacterial endotoxin (Nakamura et al., 1998; Shini et al., 2008a). The APR is a set of systemic changes mediated by pro-inflammatory cytokines and is typically manifested by a febrile reaction, a synthesis of acute phase proteins and sickness behaviour (Baumann and Gauldie, 1994). The main purpose of this reaction is to control and prevent further tissue damage and restore homeostasis (Koutsos and Klasing, 2001; Owen-Ashley and Wingfield, 2007). Moreover, the APR as a component of innate immunity represents a prominent immune defence in newly hatched birds that gain their full immunocompetence only by a few days post-hatch (Mast and Goddeeris, 1999).

The absence of a corticosterone surge after LPS treatment in the LET chicks in the present study suggests that the hormone release

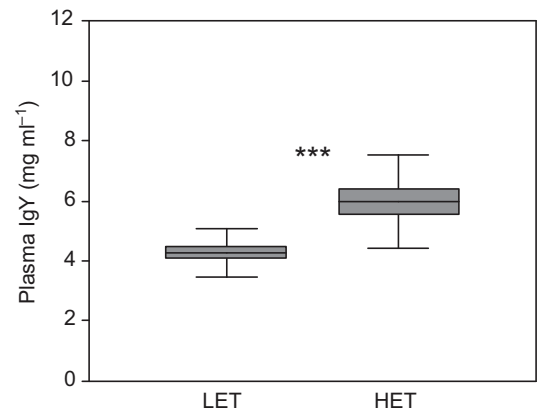


Fig. 3. Plasma IgY concentrations in 12-day-old Japanese quail selected for high (HET) and low egg testosterone (LET) content. Data are means \pm s.e.m. (shaded box) and \pm s.d. (error bars). *** $P<0.001$.

was either smaller or more transient than in the HET line. Glucocorticoid levels rise during the course of immune response, where they serve as important mediators of immune–endocrine interactions (Apanius, 1998). As such, glucocorticoids essentially play a dual role in modulation of the APR. They enhance the initiation phase by stimulating the hepatic sensitivity to pro-inflammatory cytokines and, simultaneously, they can provide negative feedback to prevent an escalation of an acute inflammatory response (Jensen and Whitehead, 1998). As plasma corticosterone concentrations decreased to the basal levels in both lines at 3 h post-LPS treatment, early high corticosterone release in HET quail provides support for the immune-promoting effects.

Corticosterone also influences the chemotaxis of circulating leukocytes, resulting in reduced levels of Ly, eosinophils, basophils and monocytes and increased levels of neutrophils (Sapolsky et al., 2000). In our study, the He:Ly ratio rose at 3 h post-immune challenge in both lines, with a significantly more pronounced response observed in the HET chicks. Indeed, this effect reflects the enhanced corticosterone stimulation in the HET line. Moreover, the increased He:Ly ratio was associated with decreased relative percentages of Ly and increased percentages of He in the circulation. Such a pattern corresponds with a stress-induced decline in leukocyte numbers that can be explained by a redistribution of leukocytes between the blood and other organs (Dhabhar, 2009). Peripheral lymphocytes decrease mainly because of their traffic to lymphoid organs (Trout et al., 1996) whereas heterophilia is caused by an increased migration of immature cells from the bone marrow and

Table 2. White blood cell (WBC) differential counts (means \pm s.e.m.) in 12-day-old quail from the high (HET) and low egg testosterone (LET) lines at 1 and 3 h post-lipopolysaccharide (LPS) challenge

WBC type	Time post-LPS challenge	LET line		HET line	
		PBS (N=15)	LPS (N=16)	PBS (N=14)	LPS (N=15)
Heterophils	1 h	32.1 \pm 5.2	44.1 \pm 5.7	35.2 \pm 3.5	37.4 \pm 5.2
	3 h	40.1 \pm 4.6	65.7 \pm 5.2	34.2 \pm 5.5	77.4 \pm 4.4
Lymphocytes	1 h	66.6 \pm 5.1	54.3 \pm 5.7	62.2 \pm 3.8	60.4 \pm 5.5
	3 h	58.0 \pm 4.3	31.3 \pm 4.4	63.6 \pm 5.7	21.0 \pm 4.5
Eosinophils	1 h	0.9 \pm 0.2	0.8 \pm 0.3	1.0 \pm 0.4	1.5 \pm 0.4
	3 h	1.3 \pm 0.4	1.8 \pm 0.8	1.2 \pm 0.5	1.0 \pm 0.6
Monocytes	1 h	0.4 \pm 0.2	0.8 \pm 0.3	1.5 \pm 0.4	0.7 \pm 0.4
	3 h	0.6 \pm 0.1	1.2 \pm 0.5	1.0 \pm 0.3	0.6 \pm 0.3

PBS, phosphate buffered saline.

a release of marginated He into the bloodstream (Harmon, 1998; Shini et al., 2008b). Thus, both measured parameters (plasma corticosterone and the He:Ly ratio) indicate a stronger response to bacterial antigen in HET compared with LET chicks. Regarding an adaptive explanation, we can assume that quail exposed to high yolk androgen levels are better to cope with immunological challenges, although prolonged corticosterone stimulation in response to chronic exposure to pathogens could result in pathological changes. However, the stronger innate response of HET quail does not predict the activity of other arms of the immune system, especially the acquired immunity, which should be studied further.

We found that the HET chicks were heavier than the LET chicks from the second week of age, indicating enhanced post-hatching growth. This is in accordance with our previous results showing accelerated growth in the HET line (Okuliarova et al., 2011a). However, data obtained by experimental injections of exogenous T into eggs showed predominantly no effects on growth in precocial birds (Andersson et al., 2004; Rubolini et al., 2006) and variable effects in altricial birds (Schwabl, 1996; Müller et al., 2008). Thus, although the adaptive significance of growth promoting effects of maternal androgens has been widely discussed (Schwabl, 1996; Groothuis et al., 2005b), recent studies suggest that the data interpretation may be complicated by sex-specific effects of yolk androgens (Saino et al., 2006; von Engelhardt et al., 2006; Sockman et al., 2008; Pitala et al., 2009), estimating an optimal dose of injected hormone in relation to its endogenous content (Navara et al., 2005; Cucco et al., 2008) and the biological potential of other hormones and substances in the egg (Hegyi and Schwabl, 2010). Our data obtained in genetic lines selected for egg T content imply that this model represents a biologically relevant way to increase maternal androgens and can simultaneously reduce the confounding effects of the above-mentioned factors.

During the chick phase, the full maturation of individual physiological traits requires increased consumption of energy and nutrient resources, leading to a possible trade-off between growth and immunity (Soler et al., 2003; Brzęk and Konarzewski, 2007). Such a linkage has also been considered between yolk-androgen-stimulated growth and its expected costs, paid by the reduced immunocompetence of offspring (Groothuis et al., 2005b). Indeed, an increase of yolk androgen levels positively affected growth and decreased the cell-mediated immune response to phytohaemagglutinin in black-headed gull (Groothuis et al., 2005a) and eastern bluebird (*Sialia sialis*) offspring (Navara et al., 2005), as well as suppressed the immune response in fast-growing Chinese painted quail (*Coturnix chinensis*) chicks (Andersson et al., 2004). However, other studies have demonstrated immunosuppressive effects of yolk androgens without any influence on the offspring's growth and *vice versa* (Müller et al., 2005; Tschirren et al., 2005; Pitala et al., 2009; Sandell et al., 2009).

As HET quail exhibited faster growth than LET quail, we predicted reduced immunocompetence in the HET line. Furthermore, the APR is a highly nutrient-demanding component of the immune system (Klasing, 2004) and a weaker inflammatory response has been demonstrated in broiler chickens in comparison with layer chickens (Leshchinsky and Klasing, 2001). Despite this prediction, we found a more intense acute phase immune response in HET chicks compared with LET chicks, indicating a positive effect of yolk androgens on innate immune defence in young quail. Similarly, egg T injections have been shown to stimulate both growth and cell-mediated immunity in house finch (Navara et al., 2006) and grey partridge chicks (Cucco et al., 2008). In the latter study, these beneficial effects were recorded only using a low T dose, whereas

a high (supra-physiological) dose of T elicited negative effects on both traits (Cucco et al., 2008). Immunoenhancement, resulting from experimentally increased yolk T levels, has also been reported for humoral immune response in adult zebra finch (Tobler et al., 2010).

Therefore, our current data do not support the suggestion that the immunosuppressive effects of maternal androgens are a consequence of redirecting energy to enhanced growth. In the present study, we examined the APR under optimal nutritional conditions and thus we cannot rule out different costs of other immune system components, especially in relation to food deprivation. Nonetheless, it is likely that there are also other mechanisms through which maternal androgens may modulate immune functions in offspring. Direct effects of yolk androgens on the immune system can be expressed through androgen receptors that are detected in the bursa of Fabricius during early embryonic development (Gasc and Stumpf, 1981). For example in chickens, embryo T treatment influenced the bursa mass in a dose-dependent manner, with positive effects of low T doses and negative effects of high T doses (Norton and Wira, 1977). However, quail from the HET and LET lines did not differ significantly in the mass of the bursa of Fabricius in our study. Regarding the line differences in plasma corticosterone response, a more probable mechanism of yolk androgen action can be explained through the changed sensitivity of the HPA axis (Groothuis and Schwabl, 2008). In line with this idea, an experimental manipulation of yolk androgen has been found to result in increased plasma corticosterone concentrations in American kestrel (*Falco sparverius*) nestlings, although the effect was associated with deleterious consequences on development (Sockman and Schwabl, 2001).

At 12 days of age, total IgY levels were higher in the circulation of HET chicks than LET chicks. For now, it is not clear whether these elevated antibody concentrations reflect higher maternal antibody transfer, enhanced activity of offspring's own immune system or a combination of both. In Japanese quail, maternal antibodies decline between 6 and 11 days post-hatching, and thus the synthesis of endogenous antibodies seems to be independently active only after this age (Grindstaff, 2008). The maternal antibodies persist in the circulation of quail chicks during the first 30 days of life (Grindstaff, 2010), and over this period they can provide passive protection against pathogenic agents the mother encountered during her prior life (Brambell, 1970), prime the humoral immune response (Gasparini et al., 2006; Reid et al., 2006) and reduce the costs of antigen challenges associated with growth suppression (Grindstaff, 2008). Current literature does not provide any evidence that maternal immunity can prime innate immune responses in offspring, and thus we can only speculate to what extent maternal antibodies are able to affect the enhanced APR in the HET quail. Moreover, increased antibody levels in our study may reflect either enhanced IgY endogenous production in young HET quail or the promoted maturation of their neonatal immune system.

In conclusion, our data demonstrate the enhanced APR to immune stressor together with elevated growth in chicks from the HET line when compared with the LET line. As maternally derived androgens in the egg possess additive genetic variance (Okuliarova et al., 2011b) and can positively affect some fitness-related traits in offspring, we expected evolutionary constraints on yolk androgen transfer in terms of immunosuppressive effects in the HET line. In contrast to our expectations, the genetic selection for high egg T content did not limit innate non-specific immunity in young quail, and even resulted in immunoenhancement effects. The responsiveness of other arms of the immune system and maternal

antibody deposition will be studied in future to generalize the effects of selection for yolk T on the immunocompetence of progeny.

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REFERENCES

- Andersson, S., Uller, T., Lohmus, M. and Sundstrom, F. (2004). Effects of egg yolk testosterone on growth and immunity in a precocial bird. *J. Evol. Biol.* **17**, 501-505.
- Apanius, V. (1998). Stress and immune defense. *Stress Behav.* **27**, 133-153.
- Baumann, H. and Gaudie, J. (1994). The acute-phase response. *Immunol. Today* **15**, 74-80.
- Brambell, F. W. R. (1970). The transmission of passive immunity from mother to young. Amsterdam: North-Holland Publishing Co.
- Brzęk, P. and Konarzewski, M. (2007). Relationship between avian growth rate and immune response depends on food availability. *J. Exp. Biol.* **210**, 2361-2367.
- Cucco, M., Guasco, B., Malacarne, G., Ottonelli, R. and Tanvez, A. (2008). Yolk testosterone levels and dietary carotenoids influence growth and immunity of grey partridge chicks. *Gen. Comp. Endocrinol.* **156**, 418-425.
- Dhabhar, F. S. (2009). Enhancing versus suppressive effects of stress on immune function: implications for immunoprotection and immunopathology. *Neuroimmunomodulation* **16**, 300-317.
- Eising, C. M. and Groothuis, T. G. G. (2003). Yolk androgens and begging behaviour in black-headed gull chicks: an experimental field study. *Anim. Behav.* **66**, 1027-1034.
- Eising, C. M., Müller, W. and Groothuis, T. G. G. (2006). Avian mothers create different phenotypes by hormone deposition in their eggs. *Biol. Lett.* **2**, 20-22.
- Gasc, J. M. and Stumpf, W. E. (1981). The bursa of Fabricius of the chicken embryo: localization and ontogenic evolution of sex-steroid target cells. *J. Embryol. Exp. Morphol.* **63**, 225-231.
- Gasparini, J., McCoy, K. D., Staszewski, V., Haussy, C. and Boulonier, T. (2006). Dynamics of anti-Borrelia antibodies in blacklegged kittiwake (*Rissa tridactyla*) chicks suggest a maternal educational effect. *Can. J. Zool.* **84**, 623-627.
- Gil, D. (2008). Hormones in avian eggs: physiology, ecology and behavior. *Adv. Stud. Behav.* **38**, 337-398.
- Grindstaff, J. L. (2008). Maternal antibodies reduce costs of an immune response during development. *J. Exp. Biol.* **211**, 654-660.
- Grindstaff, J. L. (2010). Initial levels of maternally derived antibodies predict persistence time in offspring circulation. *J. Ornithol.* **151**, 423-428.
- Grindstaff, J. L., Demas, G. E. and Ketterson, E. D. (2005). Diet quality affects egg size and number but does not reduce maternal antibody transmission in Japanese quail *Coturnix japonica*. *J. Anim. Ecol.* **74**, 1051-1058.
- Groothuis, T. G. G. and Schwabl, H. (2008). Hormone-mediated maternal effects in birds: mechanisms matter but what do we know of them? *Philos. Trans. R. Soc. Lond. B* **363**, 1647-1661.
- Groothuis, T. G. G., Eising, C. M., Dijkstra, C. and Müller, W. (2005a). Balancing between costs and benefits of maternal hormone deposition in avian eggs. *Biol. Lett.* **1**, 78-81.
- Groothuis, T. G. G., Müller, W., von Engelhardt, N., Carere, C. and Eising, C. (2005b). Maternal hormones as a tool to adjust offspring phenotype in avian species. *Neurosci. Biobehav. Rev.* **29**, 329-352.
- Harmon, B. G. (1998). Avian heterophils in inflammation and disease resistance. *Poult. Sci.* **77**, 972-977.
- Hegy, G. and Schwabl, H. (2010). Do different yolk androgens exert similar effects on the morphology or behaviour of Japanese quail hatchlings *Coturnix japonica*? *J. Avian Biol.* **41**, 258-265.
- Jensen, L. E. and Whitehead, A. S. (1998). Regulation of serum amyloid A protein expression during the acute-phase response. *Biochem. J.* **334**, 489-503.
- Kincade, P. W. and Cooper, M. D. (1971). Development and distribution of immunoglobulin-containing cells in the chicken. An immunofluorescent analysis using purified antibodies to mu, gamma and light chains. *J. Immunol.* **106**, 371-382.
- Klasing, K. C. (2004). The cost of immunity. *Acta Zool. Sinica* **50**, 961-969.
- Koutsos, E. A. and Klasing, K. C. (2001). The acute phase response in Japanese quail (*Coturnix coturnix japonica*). *Comp. Biochem. Physiol.* **128C**, 255-263.
- Leshchinsky, T. V. and Klasing, K. C. (2001). Divergence of the inflammatory response in two types of chickens. *Dev. Comp. Immunol.* **25**, 629-638.
- Mast, J. and Goddeeris, B. M. (1999). Development of immunocompetence of broiler chickens. *Vet. Immunol. Immunopathol.* **70**, 245-256.
- Mousseau, T. A. and Fox, C. W. (1998). The adaptive significance of maternal effects. *Trends Ecol. Evol.* **13**, 403-407.
- Müller, W., Groothuis, T. G. G., Kasprzik, A., Dijkstra, C., Alatalo, R. V. and Sittari, H. (2005). Prenatal androgen exposure modulates cellular and humoral immune function of black-headed gull chicks. *Proc. R. Soc. Lond. B* **272**, 1971-1977.
- Müller, W., Vergauwen, J. and Eens, M. (2008). Yolk testosterone, postnatal growth and song in male canaries. *Horm. Behav.* **54**, 125-133.
- Müller, W., Dijkstra, C. and Groothuis, T. G. G. (2009). Maternal yolk androgens stimulate territorial behaviour in black-headed gull chicks. *Biol. Lett.* **5**, 586-588.
- Nakamura, K., Mitarai, Y., Yoshioka, M., Koizumi, N., Shibahara, T. and Nakajima, Y. (1998). Serum levels of interleukin-6, alpha1-acid glycoprotein, and corticosterone in two-week-old chickens inoculated with *Escherichia coli* lipopolysaccharide. *Poult. Sci.* **77**, 908-911.
- Navara, K. J., Hill, G. E. and Mendonca, M. T. (2005). Variable effects of yolk androgens on growth, survival, and immunity in eastern bluebird nestlings. *Physiol. Biochem. Zool.* **78**, 570-578.
- Navara, K. J., Hill, G. E. and Mendonca, M. T. (2006). Yolk testosterone stimulates growth and immunity in house finch chicks. *Physiol. Biochem. Zool.* **79**, 550-555.
- Norton, J. M. and Wira, C. R. (1977). Dose-related effects of the sex hormones and cortisol on the growth of the bursa of Fabricius in chick embryos. *J. Steroid Biochem.* **8**, 985-987.
- Okuliarova, M., Kostal, L. and Zeman, M. (2011a). Effects of divergent selection for yolk testosterone content on growth characteristics of Japanese quail. *Comp. Biochem. Physiol.* **160A**, 81-86.
- Okuliarova, M., Groothuis, T. G. G., Skrobánek, P. and Zeman, M. (2011b). Experimental evidence for genetic heritability of maternal hormone transfer to offspring. *Am. Nat.* **177**, 824-834.
- Owen-Ashley, N. T. and Wingfield, J. C. (2007). Acute phase responses of passerine birds: characterization and seasonal variation. *J. Ornithol.* **148**, S583-S591.
- Partecke, J. and Schwabl, H. (2008). Organizational effects of maternal testosterone on reproductive behavior of adult house sparrows. *Dev. Neurobiol.* **68**, 1538-1548.
- Pitala, N., Ruuskanen, S., Laaksonen, T., Doligez, B., Tschirren, B. and Gustafsson, L. (2009). The effects of experimentally manipulated yolk androgens on growth and immune function of male and female nestling collared flycatchers *Ficedula albicollis*. *J. Avian Biol.* **40**, 225-230.
- Reid, J. M., Arcese, P., Keller, L. F. and Hasselquist, D. (2006). Long-term maternal effect on offspring immune response in song sparrows *Melospiza melodia*. *Biol. Lett.* **2**, 573-576.
- Rubolini, D., Romano, M., Martinelli, R., Leoni, B. and Saino, N. (2006). Effects of prenatal yolk androgens on armaments and ornaments of the ring-necked pheasant. *Behav. Ecol. Sociobiol.* **59**, 549-560.
- Saino, N., Ferrari, R. P., Romano, M., Martinelli, R., Lacroix, A., Gil, D. and Møller, A. P. (2006). Maternal allocation of androgens and antagonistic effects of yolk androgens on sons and daughters. *Behav. Ecol.* **17**, 172-181.
- Sandell, M. I., Tobler, M. and Hasselquist, D. (2009). Yolk androgens and the development of avian immunity: an experiment in jackdaws (*Corvus monedula*). *J. Exp. Biol.* **212**, 815-822.
- Sapolsky, R. M., Romero, L. M. and Munck, A. U. (2000). How do glucocorticoids influence stress responses? Integrating permissive, suppressive, stimulatory, and preparative actions. *Endocr. Rev.* **21**, 55-89.
- Schwabl, H. (1996). Maternal testosterone in the avian egg enhances postnatal growth. *Comp. Biochem. Physiol.* **114A**, 271-276.
- Shini, S., Kaiser, P., Shini, A. and Bryden, W. L. (2008a). Biological response of chickens (*Gallus gallus domesticus*) induced by corticosterone and a bacterial endotoxin. *Comp. Biochem. Physiol.* **149B**, 324-333.
- Shini, S., Kaiser, P., Shini, A. and Bryden, W. L. (2008b). Differential alterations in ultrastructural morphology of chicken heterophils and lymphocytes induced by corticosterone and lipopolysaccharide. *Vet. Immunol. Immunopathol.* **122**, 83-93.
- Sockman, K. W. and Schwabl, H. (2001). Plasma corticosterone in nestling American kestrels: effects of age, handling stress, yolk androgens, and body condition. *Gen. Comp. Endocrinol.* **122**, 205-212.
- Sockman, K. W., Weiss, J., Webster, M. S., Talbott, V. and Schwabl, H. (2008). Sex-specific effects of yolk-androgens on growth of nestling American kestrels. *Behav. Ecol. Sociobiol.* **62**, 617-625.
- Soler, J. J., de Neve, L., Perez-Contreras, T., Soler, M. and Sorci, G. (2003). Trade-off between immunocompetence and growth in magpies: an experimental study. *Proc. R. Soc. Lond. B* **270**, 241-248.
- Tobler, M., Hasselquist, D., Smith, H. G. and Sandell, M. I. (2010). Short- and long-term consequences of prenatal testosterone for immune function: an experimental study in the zebra finch. *Behav. Ecol. Sociobiol.* **64**, 717-727.
- Trout, J. M., Mashaly, M. M. and Siegel, H. S. (1996). Changes in blood and spleen lymphocyte populations following antigen challenge in immature male chickens. *Br. Poult. Sci.* **37**, 819-827.
- Tschirren, B., Saladin, V., Fitze, P. S., Schwabl, H. and Richner, H. (2005). Maternal yolk testosterone does not modulate parasite susceptibility or immune function in great tit nestlings. *J. Anim. Ecol.* **74**, 675-682.
- von Engelhardt, N., Carere, C., Dijkstra, C. and Groothuis, T. G. G. (2006). Sex-specific effects of yolk testosterone on survival, begging and growth of zebra finches. *Proc. R. Soc. Lond. B* **273**, 65-70.
- Watanabe, S. and Nagayama, F. (1979). Studies on the serum IgG level in Japanese quail. *Jpn. Poult. Sci.* **16**, 59-64.