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Diet quality affects egg size and number but does not reduce maternal antibody transmission in Japanese quail *Coturnix japonica*

JENNIFER L. GRINDSTAFF*†, GREGORY E. DEMAS* and ELLEN D. KETTERSON*

**Indiana University, Department of Biology and Center for the Integrative Study of Animal Behaviour, 1001 E. Third Street, Bloomington, IN 47405 USA*

Summary

1. The ability to resist infection is an important component of survival and lifetime reproductive success. Mounting and maintaining an immunological defence is assumed to be energetically costly and nutritional resources expended on immune function may induce trade-offs with other energetically expensive functions, including reproduction. Resource limitation may even have transgenerational effects on immune function during reproduction because mothers are the primary source of humoral immunity in young vertebrates.

2. To determine whether protein restriction affects humoral immunity, either within or across generations, we fed adult Japanese quail *Coturnix japonica* isocaloric diets containing either the recommended protein content for reproducing adults (20%), or a low protein diet (12%).

3. Females fed the low protein diet weighed less than control females and produced fewer eggs that were smaller in size. However, dietary treatment did not affect the antibody response to a novel antigen (SRBCs) or immunoglobulin concentration (IgG = IgY) in either females or their eggs.

4. This suggests that the magnitude of the humoral immune response is either not constrained by protein availability or that birds can compensate for low dietary protein intake when fed *ad libitum*. Maternal protein reserves may be catabolized to support egg production and antibody formation under protein restriction such that only very severe malnutrition would affect immune function. Future work should address whether other resources mediate the trade-off between immunity and reproduction or whether other components of the immune response are impacted by resource limitation.

Key-words: antibody transmission, immunity, maternal effects, protein, trade-off.

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Introduction

Infectious diseases exert strong selection against hosts (Grenfell & Dobson 1995); therefore, immune function may be an important determinant of survival and lifetime reproductive success (Sheldon & Verhulst 1996; Lochmiller & Deerenberg 2000). Resource limitation is thought to account for much of the variation in immune function among individuals because the vertebrate immune response is reliant upon significant supplies of proteins and amino acids (Klasing 1998). During an immune response, animals often experience negative nitrogen balance through an energy-dependent proteolytic response in skeletal muscle (Lochmiller & Deerenberg 2000). This accelerated breakdown of proteins further increases the energetic demands of infected individuals (Hasselgren & Fischer 1998). As a result, even mild immune challenges may result in negative nitrogen balance that persists for at least several days (Klasing 1998). The humoral immune response is

Correspondence: Jennifer L. Grindstaff, Department of Animal Ecology, Ecology Building, 22362 Lund, Sweden. Jennifer.Grindstaff@zooekol.lu.se
†Present address: Lund University, Department of Animal Ecology, Ecology Building, 22362 Lund, Sweden.

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presumed to require resources that an individual may otherwise have allocated to other functions (Sheldon & Verhulst 1996). Consequently, the immune response is assumed to be costly to the host in terms of increasing resource demands and to necessitate trade-offs with other resource-demanding functions. In adults, this trade-off may be manifested as a decrease in reproductive effort during an immune challenge (Råberg *et al*. 2000). In young birds, immune defence may trade off with growth (Soler *et al*. 2003). However, the effects of resource-based trade-offs across generations are less well understood. Although several studies have documented effects of reduced resource availability on cell-mediated immunity within a generation (Lochmiller, Vestey & Boren 1993; Alonso-Alvarez & Tella 2001), little research has addressed whether resource availability influences maternal antibody transmission (Chandra 1975; Sams *et al*. 1995).

The transmission of antibodies to eggs may represent a significant immunological and resource drain for ovulating females because up to 45 mg day^{-1} of IgG (sometimes referred to as IgY) may be accumulated in the yolk of hens' eggs during days −3 and −2 (day 0 = day of laying) and once mature, hen oocytes contain 100–200 mg of maternal IgG or 10–20% of the hen's steady-state level (Kowalczyk *et al*. 1985). If antibody production is limited by the availability of specific nutrients or minerals, maternal antibody transmission may be constrained by the availability of these precursors in ovulating females. Furthermore if resource availability constrains the immune response, then trade-offs between investment in life history components and immunity should be particularly evident at stages of maximal demand (e.g. reproduction), especially when resources are very limited (Landete-Castillejos *et al*. 2002).

In vertebrates, maternal physiological condition is likely to have profound effects on offspring immunity. Young vertebrates have limited ability to synthesize antibodies endogenously and thus, most of the humoral or antibody-mediated immune defence of offspring is maternally derived (Brambell 1970; Grindstaff, Brodie & Ketterson 2003). Mothers transmit antibodies to offspring either transplacentally (IgG) and in breast milk (IgA) in mammals or during yolk formation (primarily IgG) in oviparous vertebrates. The amount and types of antibodies transmitted may be determined by a variety of environmental factors, including the social environment (Saino *et al*. 2002), disease exposure (Lemke & Lange 1999; Lundin *et al*. 1999; Gasparini *et al*. 2001), and food availability (Chandra 1975; Michalek, Rahman & McGhee 1975; Roulin & Heeb 1999).

In the present study, we asked first whether dietary protein restriction might influence adult female immunoglobulin concentrations and antibody response to a novel antigen. Secondly, we asked whether maternal protein restriction has transgenerational consequences by influencing egg size or levels of antibodies in eggs. To address these questions, we fed adult female Japanese quail *Coturnix japonica* isocaloric diets containing either the recommended protein content for reproducing adults (20%) (Woodard *et al*. 1973) or a low protein diet (12%) and then quantified maternal antibody response to a novel antigen and IgG concentrations in female serum and egg yolks. We chose to manipulate dietary protein because previous research has demonstrated that dietary protein manipulation may affect immune function in young birds (Lochmiller *et al*. 1993) and protein may also be a limiting resource for egg production (Penz & Jensen 1991; Bolton, Houston & Monaghan 1992). We chose to study Japanese quail in the lab in order to isolate the specific effects of maternal protein intake on investment in reproduction and antibody production; however, our results should be applicable to more natural conditions.

Materials and methods

STUDY SPECIES

Japanese quail were obtained from a randomly bred selection line at Purdue University and were of approximately equivalent age (within 6 weeks of age of one another). Birds were housed in a quail battery breeder (Georgia Quail Farms) with 30 cages and one breeding pair per cage. Therefore, parentage of eggs was unequivocally assigned. Eggs were collected daily and the cage and date were recorded. Length, breadth, and mass of each egg at the time of collection were also recorded. Egg volume was calculated according to the formula: $V = (\pi LB^2)/6000$ where L is length and B is maximal breadth (Tatum 1975). After measurement, whole eggs were wrapped in Parafilm® and frozen at −20 °C for later analysis. We collected blood samples from females to measure IgG concentrations prior to the diet manipulation; after the diet manipulation, but prior to immunization; and 6 days post-immunization.

DIETARY MANIPULATION

After a 2-week acclimation period, adults were fed one of two isocaloric diets containing either 20% protein (control) or 12% protein (low) following the dietary protein manipulation described by Lochmiller *et al*. (1993) (Table 1). The primary protein source of the diet was isolated soy protein (90% crude protein content, Protein Technologies International, St Louis, MO, USA). The low protein diet initially contained 10% crude protein, but the protein content was increased to 12% after we found that females fed the 10% diet ceased egg production. After the increase in protein content, egg production resumed. All quail were fed the experimental diets (control or 12% protein) for 4 weeks prior to immunization.

IMMUNIZATION

We collected blood samples before immunization to screen for background levels of binding to sheep erythrocytes. Quail were then immunized via the brachial vein with

Table 1. Formulations of low protein and control diets. Diets were based on those used in Lochmiller *et al*. (1993)

Nutrient	12% protein	20% protein
Corn starch	60·2%	52·2%
Ground corn	22%	22%
Iso-soy	12%	20%
Vegetable oil	5·5%	5·5%
Dicalcium phosphate	3%	3%
Vitamin/mineral mix	0·4%	0·4%
DL-Methionine	0·45%	0·45%
Salt	0·45%	0·45%
Calcium carbonate	6%	6%

Sources of feed components: corn starch – Sysco food service suppliers; ground corn – Monroe Livestock and Pet Food Store; Iso-soy – Protein Technologies International; dicalcium phosphate – Solutia; vitamin/trace mineral mix – Dawes quail vitamin/mineral mix; methionine – Purdue University feed mill; calcium carbonate (limestone) – Monroe Livestock and Pet food store.

0·2 mL 2% washed sheep red blood cells (ICN Biochemical cat. no. 55876) (SRBCs) suspended in phosphate-buffered saline (PBS) (Boa-Amponsem, Dunnington & Siegel 1997). SRBCs are a novel antigen for quail and induce formation of antibodies in immunized individuals without causing illness (Hudson & Hay 1989). Six days post-immunization, a second blood sample was collected to assay for antibody response to SRBCs. We also quantified circulating concentrations of IgG, which includes antibodies specific to SRBCs, both before and after the diet manipulation and immunization with SRBCs.

ADULT ANTIBODY ASSAYS

IgG concentrations in females were quantified using an enzyme-linked immunosorbent assay (ELISA) (modified from Demas & Nelson 1996). ELISA plates were coated with 100 µL of antichicken IgG (donkey antichicken IgY, Jackson ImmunoResearch Laboratories (West Grove, PA, USA) product no. 703-005-155) at a concentration of 3 µg mL^{-1} suspended in carbonate buffer (0·15 M, pH 9·6). Plates were incubated overnight at 4 °C to coat. The next day, plates were blocked with 5% milk powder (Mix 'N Drink, Saco Foods Inc., Middleton, WI) diluted in PBS-Tween-20 (0·5 mL Tween-20 per litre PBS) for at least 2 h at room temperature. During the incubation, serum samples were diluted 1 : 20 000 in diluent (1% milk powder, PBS-Tween-20). After washing, diluted serum samples were added to the plate in duplicate. We also included at least two blank wells (containing diluent only) on each plate. The plates were again incubated overnight at 4 °C. On the third day, 100 µL of the labelling antibody (AP-conjugated rabbit, antichicken IgG, Sigma (Sigma-Aldrich, St Louis, MO, USA) catalogue no. A-9171) diluted 1 : 1000 in the diluent were added to every well after washing. The plates were then incubated for 1 h at 37 °C. After washing, 100 µL of substrate buffer were added to every well. The plates were then immediately transferred to a Bio-Rad (Hercules, CA, USA) Benchmark microplate reader (catalogue no. 170-6850) and read at 30-s intervals for 14 min using a 405-nm wavelength filter. All immunoglobulin concentrations are reported as the slope of the substrate conversion (in 10^{-3} × optical densities (OD); m_{od}) over time (m_{od} min^{-1}), with a higher slope indicating a higher concentration of antibodies in the sample.

We calculated the mean of the duplicate values for each sample to compute immunoglobulin concentration. Also the mean value of the blanks was subtracted from the measured immunoglobulin concentration to account for nonspecific binding. Background levels were always less than 0·2 OD units. On each plate, we included a serial dilution of a chicken-IgG standard (chicken IgY, Promega catalogue no. G116A) for a standard curve (0·1 µg mL^{-1}, 0·05, 0·025, 0·0125, 0·00625, 0·003125). We used the differences between the standard curves to account for between-plate variation.

Specific antibody responses to SRBC immunization were quantified in maternal serum using a haemagglutination assay (Hudson & Hay 1989). Briefly, the complement in 20 µL of each serum sample was heat-inactivated at 56 °C for 30 min. These serum aliquots were diluted 1 : 1 in PBS and serially diluted in 96-well round-bottomed microtitre plates (Supplier: Nunc). Twenty microlitres of 2% washed SRBCs were added to each well, and the plates were incubated at 37 °C for 60 min. The agglutination titre was expressed as the log of the reciprocal of the lowest dilution showing agglutination (Hudson & Hay 1989).

EGG ANTIBODY ASSAYS

To quantify IgG levels in egg yolks, we used a similar ELISA procedure as described above for serum samples. Because egg albumin thaws more quickly than yolk, eggshells and albumin of frozen eggs were easily peeled away from the yolk. Any remaining albumin and the yolk membranes were removed by rolling the yolk on absorbent paper. Next yolks were homogenized by mixing with a spatula and 0·2 g of homogenized yolk were added to 1 mL of PBS-T. Diluted yolks were vortexed thoroughly with glass beads to facilitate mixing. The diluted yolk samples were then used in the ELISA assay described above at a dilution of 1 : 25 000. We quantified IgG levels in eggs laid both before and after the maternal diet manipulation, but all of the eggs within each time period were laid over the course of 3 days. The post-diet eggs were laid approximately 14 days after maternal immunization and 6 weeks after maternal diet manipulation. We analysed eggs from this time period only to control for the effect of time elapsed since immunization and diet manipulation.

Anti-SRBC antibody titres in egg yolks were quantified using the haemagglutination assay after first removing lipids from the yolks. First, frozen yolks were separated from the albumin and shell as described above and weighed. A 1-g sample of homogenized egg yolk was then added to 5 mL of Tris-buffered saline (TBS,

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Table 2. Effects of dietary protein restriction on egg size and IgG concentrations in female serum and egg yolks. Amount of IgG in egg is the concentration of IgG in the egg yolk multiplied by the yolk mass

Response variable	LP Pre-diet	LP Post-diet	CP Pre-diet	CP Post-diet	d.f.	*F*	*P*
Egg mass	8·90 ± 0·22	8·41 ± 0·13	8·67 ± 0·15	8·92 ± 0·13	29	10·94	0·003
Egg length	29·80 ± 0·31	29·12 ± 0·26	29·60 ± 0·29	29·90 ± 0·24	30	4·58	0·04
Egg width	23·08 ± 0·21	22·69 ± 0·14	22·88 ± 0·15	23·05 ± 0·12	30	4·64	0·04
Egg volume	8·33 ± 0·19	7·87 ± 0·14	8·14 ± 0·15	8·37 ± 0·12	30	9·67	0·004
Yolk mass	2·57 ± 0·15	2·41 ± 0·16	2·39 ± 0·09	2·48 ± 0·12	18	3·08	0·096
Female IgG concentration	10·56 ± 1·46	10·11 ± 1·24	13·47 ± 0·96	10·71 ± 0·89	24	2·24	0·15
Egg IgG concentration	3·27 ± 0·63	3·93 ± 0·56	4·31 ± 0·49	4·35 ± 0·50	18	1·35	0·26
Amount of IgG in egg	8·04 ± 1·45	9·23 ± 1·30	9·99 ± 1·01	10·51 ± 1·16	18	0·33	0·57

Mean ± 1 SE, LP = low protein diet, CP = control protein diet.

pH 7·4). The mixture was thoroughly vortexed and centrifuged at 2000 *g* for 20 min at room temperature. After centrifugation, 120 µL of dextran sulphate (10% w/v; Pharmacia Biotech AB (Piscataway, NJ, USA) Average MW = 500 000) per millilitre of supernatant were added. This solution was again vortexed and incubated at room temperature for at least 30 min. Next, 50 µL of 1 M calcium chloride per millilitre of supernatant were added and the mixture was incubated for a further 30 min. After the second incubation, the solution was centrifuged for 30 min at 2000 *g* and the supernatant was removed from the pelleted lipids. The procedure was repeated for a second purification of the supernatant with one-half the amount of dextran sulphate and calcium chloride. This protein supernatant, which contained IgG, was stored at 4 °C with 10 µL of 15 mM sodium azide added as a preservative (Jensenius & Koch 1997). To determine SRBC-specific antibody titre, 20 µL of PBS were added to each well of a 96-well round-bottomed microtitre plate. Next 50 µL of the diluted supernatant were added to the first well of each sample and mixed with the PBS. Fifty microlitres of this mixture were then serially diluted across wells. Plates were incubated at 37 °C for 60 min and titres were scored as above. As described above for IgG assays, we analysed only eggs laid during a 3-day time period, approximately 14 days after maternal immunization and 6 weeks after maternal diet manipulation. Because females were immunized with SRBCs after the diet manipulation, there are no pre-diet measures of SRBC-specific response.

Statistics

For the analyses of the effect of maternal diet on egg size, the measurements from all of the eggs laid by a female at least 1 month after the diet manipulation had begun and before the end of the experiment (a period of 16 days) were averaged to generate one data point for each female. Over this time period, females laid between four and 14 eggs. This period also encompasses only eggs laid by females after the final diet modification (from 10% protein to 12% protein for the low protein diet). Owing to small serum samples and lack of egg samples during the appropriate time period for some individuals, the sample sizes for antibody analyses are not always consistent.

Results

Female body mass and investment in eggs

Females fed the low protein (LP) diet weighed significantly less than females fed the control protein (CP) diet (LP female mass = 119·2 ± 3·79 SE, CP female mass = 139·3 ± 2·74; $t = 4{\cdot}39$, d.f. = 30, $P < 0{\cdot}001$). Within females, the change in egg mass, length, width and volume from before the diet manipulation to after the diet manipulation was significantly affected by treatment (Table 2; repeated measures ANOVA, diet*time: mass: $F_{1,29} = 10{\cdot}94$, $P = 0{\cdot}003$; length: $F_{1,30} = 4{\cdot}58$, $P = 0{\cdot}04$; width: $F_{1,30} = 4{\cdot}64$, $P = 0{\cdot}04$; volume: $F_{1,30} = 9{\cdot}67$, $P = 0{\cdot}004$). Yolk mass was nearly significantly affected by the diet manipulation (Table 2; repeated measures ANOVA, diet*time: $F_{1,18} = 3{\cdot}08$, $P = 0{\cdot}096$). Finally, LP females laid eggs less frequently than CP females (Kolmogorov–Smirnov $D_{117,47} = 0{\cdot}297$, $P < 0{\cdot}05$).

Female IgG concentrations

Serum IgG concentrations in females measured before and after diet manipulation were positively correlated ($r = 0{\cdot}45$, $P = 0{\cdot}029$, $n = 24$). However, IgG concentrations in females were not affected by diet, before or after immunization (Table 2; repeated measures ANOVA, pre-diet; post-diet, pre-immunization, and post-immunization IgG concentrations: $F_{2,40} = 0{\cdot}83$, $P = 0{\cdot}45$). Serum IgG concentration also was not related to female body mass at the time of immunization ($r = 0{\cdot}21$, $P = 0{\cdot}3$, $n = 25$) or the number of eggs laid by a female ($r = 0{\cdot}15$, $P = 0{\cdot}48$, $n = 25$).

Female antibody response to a novel antigen

No pre-immunization blood samples of females contained detectable levels of anti-SRBC antibodies. Of the immunized quail, 27% ($n = 8$) did not form detectable

Table 3. Effects of dietary protein restriction on antibody titres in female serum and egg yolks. SRBC response is a measure of antibody response to a novel antigen, sheep red blood cells. SRBC titre, egg yolk*yolk mass is the SRBC-specific antibody response measured in eggs multiplied by the yolk mass

Response variable	LP	CP	d.f.	*t*	*P*
SRBC titre, female serum	6·00 ± 0·43	5·78 ± 0·31	15	0·42	0·68
SRBC titre, egg yolk	3·64 ± 0·14	3·96 ± 0·14	17	1·44	0·17
SRBC titre, egg yolk*yolk mass	9·36 ± 0·58	10·69 ± 0·57	17	1·52	0·15

Mean ± 1 SE, LP = low protein, CP = control protein.

levels of antibodies in response to the immunization; therefore, these individuals were excluded from subsequent analyses. Removing these individuals from analyses did not qualitatively affect the results. Furthermore, the proportion of nonresponders did not vary by treatment group (27% low protein females, 28% control protein females). Antibody responses to SRBCs in females after immunization were not significantly affected by protein restriction (Table 3, *t*-test: $t = 0·42$, d.f. = 15, $P = 0·68$). Female antibody response to SRBCs was positively correlated with body mass at the time of immunization ($r = 0·529$, $P = 0·03$, $n = 17$). Serum IgG concentrations and anti-SRBC antibody titres were positively correlated in females ($r = 0·45$, $P = 0·023$, $n = 25$). As observed for IgG concentrations, the number of eggs laid by a female during the experiment was not related to SRBC antibody response ($r = 0·16$, $P = 0·53$, $n = 17$).

IgG concentrations in eggs

Female and egg IgG concentrations were significantly positively correlated both before diet manipulation ($r = 0·5$, $P = 0·012$, $n = 24$) and after diet manipulation ($r = 0·74$, $P < 0·0001$, $n = 24$). Within a female, the immunoglobulin concentrations in eggs laid before diet manipulation were positively correlated with immunoglobulin concentrations in eggs laid after diet manipulation ($r = 0·76$, $P < 0·0001$, $n = 20$). As found for immunoglobulin concentrations in female circulation, IgG concentrations in eggs were not detectably affected by maternal diet (Table 2; repeated measures ANOVA, pre- and post-diet IgG concentrations: $F_{1,18} = 1·35$, $P = 0·26$). There was also no significant effect of maternal diet on the total amount of IgG in egg yolks (Table 2; repeated measures ANOVA, pre- and post-diet IgG concentrations*yolk mass: $F_{1,18} = 0·33$, $P = 0·57$). Furthermore, the laying order of the sampled egg did not affect IgG concentration ($r = -0·2$, $P = 0·42$, $n = 19$). There was no relationship between egg mass and egg IgG concentration ($r = -0·1$, $P = 0·63$, $n = 25$). Egg IgG concentration was also not influenced by female body mass ($r = -0·02$, $P = 0·91$, $n = 25$).

Antigen-specific antibodies in eggs

All egg yolks contained detectable levels of SRBC-specific antibodies. SRBC-specific antibody titre in the egg was not related to the mass of the yolk ($r = 0·28$, d.f. = 19, $P = 0·24$) and egg SRBC antibody titre was unrelated to the antibody titre measured in females 6 days after immunization ($r = -0·014$, d.f. = 17, $P = 0·96$). There was no difference in anti-SRBC antibody titre between eggs laid by low protein and control females (Table 3, *t*-test: $t = 1·44$, d.f. = 17, $P = 0·17$). The total amount of anti-SRBC antibodies did not differ by maternal diet (Table 3; anti-SRBC antibody titre*yolk mass; *t*-test: $t = 1·52$, d.f. = 17, $P = 0·15$). Diet also did not influence the proportion of IgG antibodies that were specific to SRBCs ($F_{1,19} = 2·21$, $P = 0·16$). As observed for IgG concentration, laying order of the analysed egg did not affect SRBC-specific antibody titre in the egg ($r = -0·051$, $P = 0·84$, $n = 19$). In eggs, SRBC specific antibody titres were not correlated with IgG levels ($r = -0·3$, $P = 0·21$, $n = 19$). Female body mass also was not related to antibody titre in the egg ($r = 0·21$, d.f. = 19, $P = 0·4$).

Discussion

Maternal dietary protein restriction affected both female body mass and investment in reproduction. The significant decrease in egg size in particular, may have important transgenerational implications for offspring. Furthermore, the observation that females fed a 10% protein diet did not produce eggs demonstrates that the dietary manipulation was sufficient to inhibit reproductive function. However, protein restriction had no detectable effect on immunoglobulin concentrations or antigen-specific antibody titres in adult female Japanese quail. We also did not find evidence that reduced protein intake by females depresses maternal antibody transmission to offspring. Given that antibody responses to SRBCs peak approximately 6 days post-immunization (Shimizu *et al.* 2004) and most maternal antibodies are deposited in the egg yolk within 6 days prior to ovulation (Grindstaff *et al.* 2003), any treatment-based difference in maternal antibody levels, particularly IgG concentrations, should have been detectable in eggs collected 14 days post-immunization. Although we found a significant positive correlation between immunoglobulin concentrations in maternal circulation and immunoglobulin concentrations in eggs, SRBC antibody titres in maternal circulation and eggs were not significantly correlated. There are two likely explanations for this result. First, the primary antibody response to SRBCs is composed mainly of IgM molecules that are

not readily incorporated into the egg yolk (Rose, Orlans & Buttress 1974). Although maternal antibody transmission clearly occurred as demonstrated by the presence of antibodies to SRBCs in eggs and the significant positive correlation between female and egg IgG concentrations, antibody titres in egg yolk were generally lower than in female serum and there was little variation among individual eggs. Second the ELISA method we used to quantify IgG concentrations is more sensitive than the haemagglutination method and we were therefore able to detect smaller differences among individual samples, both serum and egg samples, using the ELISA.

Other research on female ungulates fed restricted diets (calorie and/or protein restricted) has found similar results. Although fawns born to protein malnourished white-tailed deer *Odocoileus virginianus* weighed less than fawns born to control does, the concentration of maternal IgG was unaffected by maternal diet (Sams *et al.* 1995). In Iberian red deer *Cervus elaphus hispanicus*, females fed a calorie-restricted diet weighed less and produced less milk with less milk fat, protein and lactose than control females. The offspring of calorie-restricted mothers consequently exhibited smaller weight gains than control offspring (Landete-Castillejos *et al.* 2002). Mothers did not differ in immunoglobulin concentration. Surprisingly, however, offspring of calorie-restricted mothers had higher immunoglobulin levels than the offspring of control mothers (Landete-Castillejos *et al.* 2002). The authors interpreted this effect as evidence that the offspring of calorie-restricted mothers were more likely to be ill than the offspring of control mothers due to their reduced condition.

Collectively the results reported here support the thesis that the costs of supplying the immune system with the substrates and energy necessary for antibody production are low relative to the nutrient and energetic costs of reproduction or growth (Klasing & Leshchinsky 1999). Alternatively, birds on protein-restricted diets fed *ad libitum* may be able to overcome protein deficiencies by increasing total food intake and protein production. Even when females are producing maximal levels of IgG prior to reproduction, IgG synthesis still represents less than 0·05% of body weight per day (Klasing & Leshchinsky 1999). Although antibody production, *per se*, may not require large amounts of protein, the anorexia, fever and changes in nutrient use that occur after exposure to replicating antigens are likely to require substantial amounts of protein (Hasselgren & Fischer 1998; Klasing & Leshchinsky 1999; Lochmiller & Deerenberg 2000). If a female is both protein-restricted and infected by a replicating pathogen, antibody production and transmission may be constrained. In this study, we challenged females with a nonreplicating antigen to determine whether antibody production alone, without sickness behaviours, is constrained by protein availability. We found that the antibody response (at least to this nonreplicating antigen) is not constrained by protein availability, even during reproduction when protein demands are highest.

Although the costs of IgG production for breeding females may be low, the benefits of IgG transmission to offspring can be substantial. In chickens, adult levels of immunocompetence are often not present until several months after hatching (Apanius 1998; Klasing & Leshchinsky 1999). During this period, chicks are dependent upon maternal antibodies for humoral immune protection (Brambell 1970). Both the quantity and diversity of antibodies transferred by females may be important determinants of offspring survival. Offspring with higher initial levels of maternal antibodies also retain maternal antibodies in circulation for a longer period of time than young with low initial levels of maternal antibodies (Goddard, Wyeth & Varney 1994; Nicoara *et al.* 1999; Caceres, Strebel & Sutter 2000). Consequently, offspring with high initial levels of maternal antibodies will have passive immune protection for a longer period of time during the development of their own immune system. Chicks with a broader diversity of maternal antibodies are able to resist a broader array of pathogens than chicks with a low diversity of maternal antibodies (Heller *et al.* 1990; Leitner *et al.* 1994). The combined effects of low production costs for females and substantial survival benefits to females and offspring are likely to have favoured the preservation of antibody production in reproducing females even during severe protein restriction.

Protein limitation may also influence offspring immunity indirectly through a reduction in transmission of other egg components. Previous research suggests that both egg size and egg contents have important consequences for offspring fitness (Williams 1994; Bernardo 1996; Wilson 1997; Styrsky, Eckerle & Thompson 1999). For example, the level of carotenoids in the maternal diet has been demonstrated to influence both carotenoid and antibody levels in eggs (Blount *et al.* 2002; Koutsos *et al.* 2003). In turn, circulating carotenoid levels have been found to affect immune responses (Blount *et al.* 2003; Faivre *et al.* 2003). Finally, the level of yolk carotenoids has been shown to affect offspring immunity (Koutsos *et al.* 2003; Saino *et al.* 2003). Protein restriction may also affect the development of the offspring immune system (Chandra 1975; Lochmiller *et al.* 1993) or the ability of offspring to absorb maternal antibodies (Quigley & Drewry 1998). Either of these would lengthen the period of time when offspring have low antibody levels.

Protein restriction may also have stronger effects on either innate or cell-mediated immunity than on humoral immunity. Protein availability has been demonstrated to affect cell-mediated immune function in young bobwhite quail *Colinus virginianus* (Lochmiller *et al.* 1993). Depressed cell-mediated immune responses have also been observed in chickens fed amino acid deficient diets (Tsiagbe *et al.* 1987). Future studies should assess whether maternal protein restriction influences other components of the maternal and offspring immune response. Although the reduced egg size of protein-restricted mothers may indirectly affect offspring

immune function, maternal protein restriction did not significantly affect antibody transmission to eggs.

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