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The metabolic cost of avian egg formation: possible impact of yolk precursor production?

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Summary

Little is known about the energy costs of egg production in birds. We showed in previous papers that, during egg production, European starlings (*Sturnus vulgaris*) undergo a 22% increase in resting metabolic rate (RMR) and that the maintenance and activity costs of the oviduct are responsible for 18% of the variation in elevated laying RMR. Therefore, other energy-consuming physiological mechanisms must be responsible for the remaining unexplained variation in elevated laying RMR. Yolk precursor [vitellogenin (VTG) and very-low-density lipoprotein (VLDL)] production is likely to be costly because it signifies a marked increase in the biosynthetic activity of the liver. We documented the pattern of yolk precursor production in response to daily injections of 17β-estradiol (E_2) in zebra finches (*Taeniopygia guttata*). Based on this pattern we carried out an experiment in order to evaluate the metabolic costs of producing VTG and VLDL. Our E_2 treatment resulted in a significant increase in plasma VTG and VLDL levels within the natural breeding range for the species. Although RMR was measured during the period of active hepatic yolk precursor production, it did not differ significantly within individuals in response to the treatment or when comparing E_2-treated birds with sham-injected birds. This could mean that yolk precursor production represents low energy investment. However, we discuss these results in light of possible adjustments between organs that could result in energy compensation.

Key words: egg production, energy cost, resting metabolic rate, RMR, vitellogenin, very-low-density lipoprotein, zebra finch, *Taeniopygia guttata*, yolk precursor production.

Introduction

Physiological mechanisms underlying one of the major assumptions of life history theory, namely that an increased effort in current reproduction may have a negative impact on future reproductive success, are not well understood (Stearns, 1992). One reason for this lack of knowledge is that, in avian systems, most of the research attention has been focused on manipulations of reproductive effort at the incubation or chick-provisioning stages (Monaghan and Nager, 1997) and, thus, the potential physiological costs incurred earlier in breeding, i.e. during follicle development and egg production, have received very little attention. Recent research, however, has shown that the energy cost of egg production in birds may be significant (Nilsson and Raberg, 2001; Vézina and Williams, 2002). We recently showed that the physiological process of egg formation in female European starlings (*Sturnus vulgaris*) is responsible for a 22% increase in resting metabolic rate (RMR) in laying individuals (Vézina and Williams, 2002). We further demonstrated that 18% of the variation in elevated laying RMR was explained by the maintenance and activity cost of the working oviduct (Vézina and Williams, 2003) and emphasized that this organ is probably costly enough that selection has led to a very tight size–function relationship, explaining its rapid pattern of recrudescence and regression. However, 82% of the variation in laying RMR remains unexplained, suggesting that other energy-consuming physiological mechanisms must be responsible, at least in part, for the metabolic cost of egg production (Vézina and Williams, 2003).

Another component of egg production that is likely to be energetically costly is the increased liver activity involved in protein and lipid production for oogenesis. During the process of egg formation, the hypothalamus initiates a hormonal cascade by releasing gonadotropin-releasing hormone (GnRH), which induces the release of follicle-stimulating hormone (FSH) and luteinizing hormone (LH) from the pituitary gland (Williams, 1998; Scanes, 2000). These hormones stimulate the ovary to produce estrogens (Williams, 1998), which then trigger the production of the egg-yolk precursors, vitellogenin (VTG) and yolk-targeted very-low-density lipoprotein (VLDLy), by the liver (Bergink et al., 1974; Deeley et al., 1975; Wallace, 1985; Walzem, 1996; Williams, 1998), which are then secreted into the blood. During rapid yolk development, plasma VTG and VLDLy are taken up by the ovary and are processed within the follicles into yolk, the nutrient and energy source for the developing avian embryo

(Bernardi and Cook, 1960; Stifani et al., 1988; Wallace, 1985). In laying domestic hens (*Gallus gallus domesticus*), approximately 50% of the liver's daily protein synthesis is attributed to VTG production, potentially tripling the amount of protein in circulation (Gruber, 1972). Hepatic lipid production also increases markedly during this time (from 0.5–1.5 μmol triglyceride ml^{-1} plasma in non-breeders to 20–50 μmol triglyceride ml^{-1} plasma in laying hens; Griffin and Hermier, 1988), as VLDL synthesis shifts from the exclusive production of non-laying, generic VLDL to an increase in the hepatic synthesis of estrogen-dependent VLDLy (Walzem, 1996; Walzem et al., 1999). The presence of circulating VLDLy represents a dramatic shift in lipid metabolism as the structure and function of plasma VLDL particles change from larger, generic VLDL, which are involved in triglyceride (i.e. energy) transport within an individual, to smaller VLDLy, which supply the yolk with energy-rich lipid (Walzem, 1996; Walzem et al., 1999). These changes in protein and lipid metabolism are likely to be energetically costly as they are associated with an increase in the activity of the liver and potentially other organs involved in reproduction, such as the ovary.

However, in female starlings, there is no relationship between laying RMR and lean dry liver mass or plasma levels of the yolk precursors in individuals having 1–5 yolky follicles left to ovulate (Vézina and Williams, 2003). Nevertheless, this does not mean that yolk precursor production is not energetically costly. Liver mass *per se* may not be representative of the liver's metabolic intensity, i.e. the amount of energy consumed per unit tissue mass. Furthermore, the elevated RMR reported in laying starlings was measured during active laying when yolk precursor levels were already maintained at an elevated level (Challenger et al., 2001; Vézina and Williams, 2003). It is possible that laying female plasma is saturated with yolk precursors, with minimal rate of production by the liver and therefore no relationship with RMR. Alternatively, the elevated precursor levels may be the result of a balance between high hepatic production and high ovary uptake rate. Consequently, comparing precursor levels with RMR in active layers may be problematic and potentially misleading.

To measure the metabolic cost of yolk precursor production accurately, one has to measure the animal's metabolic rate at a time when the liver is known to be actively involved in VTG and VLDLy synthesis. In the present study, we documented the pattern of yolk precursor production in zebra finches (*Taeniopygia guttata*) in response to daily injections of 17β-estradiol (E_2) and measured the potential metabolic cost of VTG and VLDL production by respirometry. Zebra finches represent a very good model species for this type of study because: (1) ongoing work in our lab has shown that mass-corrected RMR is ~26% higher at the one-egg stage than at the non-breeding stage (F. Vézina, K. G. Salvante and T. D. Williams, unpublished), which is very similar to reported values for starlings (Vézina and Williams, 2002) and great tits (*Parus major*; Nilsson and Raberg, 2001); (2) the pattern of yolk precursor production during the laying cycle is known and similar to the one reported in starlings (Salvante and Williams, 2002; Challenger et al., 2001) and (3) this species responds in a known manner to E_2 injections (Williams and Martyniuk, 2000). We used E_2 doses adjusted to generate plasma yolk precursor levels within the normal range for breeding females at the peak of investment (one-egg stage; Williams and Ternan, 1999). We then measured RMR in dosed individuals during the period of known hepatic activity. In order to evaluate the metabolic cost of yolk precursor production, we compared this RMR value with the metabolic rate previously measured in all individuals.

Materials and methods

Animal care

All birds (*Taeniopygia guttata* Gould) were maintained in controlled environmental conditions (temperature 19–23°C; humidity 35–55%; constant light schedule, 14 h:10 h light:dark with lights on at 07:00 h). All individuals were housed in cages (61 cm×46 cm×41 cm) containing non-breeding, same-sex groups. All finches were maintained on a mixed-seed diet (Panicum and white millet, 50:50, approximately 12.0% protein, 4.7% lipid; Jamieson's Pet Food, Vancouver, Canada), water, grit and cuttlefish bone (calcium) *ad libitum* and received a multivitamin supplement in the drinking water once per week. All experiments and animal husbandry were carried out under a Simon Fraser University Animal Care Committee permit (558B), following the guidelines of the Canadian Committee on Animal Care.

Estradiol treatment

We know from previous studies that administration of E_2 to female zebra finches triggers partial development of the oviduct (Williams and Martyniuk, 2000). Because the maintenance and activity costs of the oviduct have previously been shown to be related to metabolic rate (Chappell et al., 1999; Vézina and Williams, 2003), we avoided any confounding effects in our results by using male zebra finches as models for this experiment. Males react to E_2 injections by producing yolk precursors in the same fashion as females (Bergink et al., 1974; Follett and Redshaw, 1974). Various E_2 doses were tested (F. Vézina, K. G. Salvante and T. D. Williams, unpublished results) in order to generate a response in plasma yolk precursor levels comparable with normal female breeding values. The final dose used in this study was 1.5 μg g^{-1} (assuming a mean mass of 17 g for all birds) of E_2 dissolved in corn oil (No Name Pure Corn Oil, Toronto, Canada).

Experiment 1: pattern of yolk precursor plasma levels in response to daily E_2 injections

In order to determine the best possible timing of RMR measurement in relation to the estradiol treatment, we had to document the rise in circulating yolk precursor levels in response to the E_2 treatment. Only VTG was monitored, since

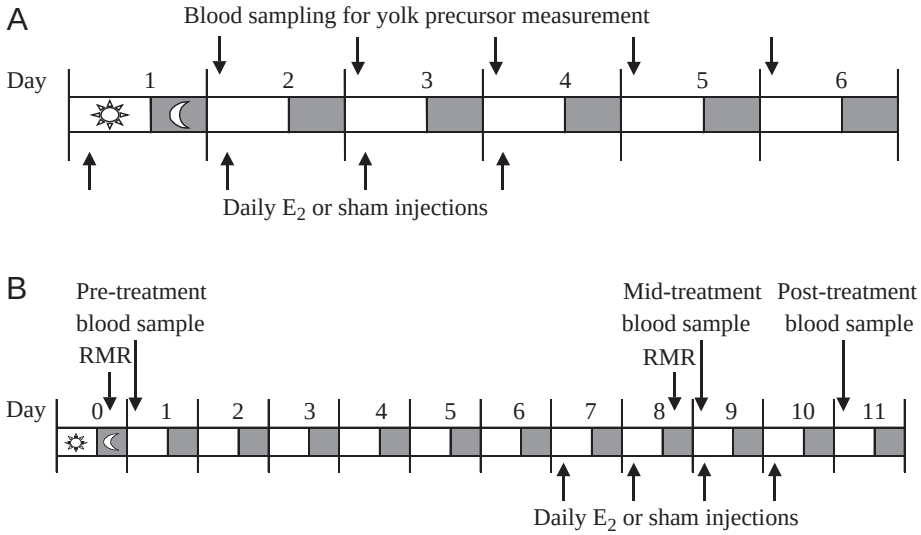

Fig. 1. (A) Experimental estradiol injection protocol to determine the pattern of vitellogenin production in response to the treatment. (B) Experimental injection and resting metabolic rate (RMR) protocol to determine the potential metabolic cost of yolk precursor production. The numbers represent the days into the treatment, with the white sections symbolizing daytime and dark sections symbolizing night-time. The arrows represent either RMR measurement, injection or blood sampling time.

both precursors have been reported to respond similarly to E_2 injections in zebra finches (Williams and Martyniuk, 2000). For this experiment, 32 male zebra finches were used. The birds were divided into five groups, and all birds received a daily E_2 injection (30 μl, i.m.) over four consecutive days (days 1–4; Fig. 1A). Starting from the day following the first injection, one group of birds was blood sampled each day until two days following the last injection (sample sizes were 4, 9, 5, 10 and 4 for days 2–6, respectively; Fig. 1A). All birds were blood sampled only once from the brachial vein. We also repeated this experiment using females (*N*=23) to confirm that both sexes responded the same way to the estradiol treatment.

Experiment 2: metabolic costs of yolk precursor production

For this experiment, we used 32 males randomly assigned to one of two groups: E_2 (injected with E_2 in corn oil; *N*=16) and sham (corn oil only; *N*=16). We used a repeated measures design where each bird was used as its own control in order to monitor changes in RMR due to E_2 administration within a bird. The experiment lasted 11 days and proceeded as follows (Fig. 1B): on the night preceding day 1, all birds had their RMR measured by respirometry (protocol described below) and were blood sampled the following morning (day 1). These data will be referred to as 'pre-treatment'. Estradiol and sham injections (30 μl, i.m.) started the morning of day 7 and lasted four consecutive days until day 10. Results from experiment 1 led us to measure RMR on the night following the second day of injections, i.e. day 8 (see justification in the Results section), and all birds were blood sampled again the following morning (day 9), from here on referred to as 'mid-treatment'. The injections were then continued until day 10 to ensure that the liver was actively producing yolk precursors at the time of RMR measurement (i.e. no plateau in precursor levels). This was confirmed by a final blood sample on day 11, from here on referred to as 'post-treatment'.

Measurement of resting metabolic rate

Blem (2000) defines basal metabolic rate (BMR) as the energy consumed by a post-absorptive bird during the resting phase of the circadian cycle at a temperature within the thermoneutral range for the animal. By definition, BMR is the lowest measurable consumption of oxygen uptake. Because birds in the second part of experiment 2 were artificially stimulated to produce yolk precursors, we considered them to be in an 'active physiological state' that may induce elevated levels of energy consumption. Therefore, although the first set of metabolic rate measurements (i.e. pre-treatment) may fall under Blem's BMR definition (last meal at least 3 h before beginning of measurements), we use the term *resting* metabolic rate throughout the paper for convenience (note that all of our measurements were taken at thermoneutrality, which is often not the case for resting metabolism reported in the literature; Blem, 2000). All RMR measurements were completed using a flow-through respirometry system (Sable Systems International, Henderson, NV, USA). Birds were taken from their cages, their body mass was measured (±0.1 g) and they were placed randomly in one of four metabolic chambers (1.5 liter) for one hour prior to the beginning of RMR measurements. All chambers continuously received approximately 500 ml min^{-1} of dry CO_2-free air (using Dryrite™ and ascarite™ as scrubbers) and were kept in the dark at 35°C, which is within the thermoneutral zone for this species (lower critical temperature = 33°C; Meijer et al., 1996). RMR measurements were always started at 00:00 h. Our setup consisted of four metabolic chambers connected to a divided air line with a valve multiplexer that allowed us to sample air coming from either ambient baseline air (scrubbed for water and CO_2) or from one metabolic chamber at a time. The air was then passed through a mass flow valve (Sierra Instruments, Monterey, CA, USA) for proper air flow reading (STP corrected) and through CO_2 and oxygen analyzers (model CA-1 and FC-1, respectively; Sable Systems International; air was water scrubbed before CO_2 analyzer, and water and CO_2 scrubbed before O_2 analyzer). All measurement sequences started by recording 10 min of baseline air. After baseline recording, the multiplexer switched, and the out-flowing air

from the first chamber was sampled for 55 min. Then the system switched back to baseline for 10 min before changing again to the second, third and fourth chambers. Preliminary analysis showed that measuring RMR using this protocol did not generate a time effect (*sensu* Hayes et al., 1992) on RMR ($F_{3,15}$=0.48, P=0.7). The birds stayed in their chambers for approximately 5 h. After RMR measurement, the birds were weighed for a second time and the average of first and second masses was used in subsequent analysis. To calculate RMR, a running mean representing 10 min of recording was passed through the data for each bird, with the lowest mean taken as RMR.

Yolk precursor analysis

In order to measure circulating levels of VTG and VLDL, blood samples were centrifuged at 2200 ***g*** for 10 min, and the plasma portion of each sample was isolated. Plasma samples were then assayed for vitellogenic zinc (Zinc kit; Wako Chemicals, Richmond, VA, USA) using the method developed for the domestic hen (Mitchell and Carlisle, 1991) and validated for passerines (Williams and Christians, 1997; Williams and Martiniuk, 2000; Challenger et al., 2001; Salvante and Williams, 2002). The concentration of vitellogenic zinc is proportional to circulating levels of VTG (Mitchell and Carlisle, 1991). The overall inter-assay coefficient of variation for the vitellogenic zinc assay (calculated from repeated analyses of a reference sample) was 14.2% (N=9 assays).

Circulating VLDL was assessed by measuring plasma triglyceride levels (Triglyceride E kit; Wako Chemicals) according to the method of Mitchell and Carlisle (1991). Plasma triglyceride has commonly been measured in non-domesticated birds as an index of total plasma VLDL, which consists of both the generic and yolk-targeted forms of VLDL (Williams and Christians, 1997; Williams and Martyniuk, 2000; Challenger et al., 2001). Despite the marked increase in circulating lipid levels reported during egg production (Griffin and Hermier, 1988; Walzem et al., 1994, 1999; Walzem, 1996), *in vivo* studies on laying poultry hens have detected only low circulating levels of intermediate-density and low-density lipoproteins, both by-products of the metabolism of generic VLDL, suggesting that VLDLy is resistant to metabolism by laying hens (Hermier et al., 1989; Walzem et al., 1994; Walzem, 1996). These studies provide evidence that the marked increase in total VLDL during avian egg production or following estrogen administration is the result of increased synthesis of the estrogen-dependent VLDLy component of total VLDL. The overall inter-assay coefficient of variation for the triglyceride assay (calculated from repeated analyses of a reference sample) was 10% (N=5 assays). All assays were run using 96-well microplates and measured using a Biotek 340i microplate reader (Winooski, VT, USA).

Statistical analysis

All data were tested to ensure normality (Shapiro–Wilk test; Zar, 1996). Plasma yolk precursor levels violated normality for both the production pattern experiment and the RMR experiment. Vitellogenin data from the first experiment were log transformed (adding 0.001 to all data to eliminate zeros) to achieve normality. Analysis of the precursor production pattern was therefore carried out on log-transformed data using standard parametric methods (see below). For clarity, yolk precursor production pattern data are presented non-transformed in Fig. 2. By contrast, log transformation of VTG and VLDL data for the RMR experiment did not result in normally distributed data. Therefore, non-parametric tests were performed (see below) when the analysis included VTG or VLDL data. For all other analysis, standard parametric statistics were used since all other variables were found to be normally distributed.

Contrary to the results of preliminary testing (see above), there was a significant time effect on RMR in experiment 2 when controlling for body mass (pre-treatment: $F_{3,27}$=2.99, P<0.05; mid-treatment: $F_{3,26}$=3.57, P<0.05). This effect translated into a decreasing RMR over time, but with a difference of less than 10% between measurements for either the first or last chamber relative to the mean of all chambers. Overall, pre- and mid-treatment RMR of birds in the first metabolic chamber was, respectively, 3.04 ml O_2 h^{-1} (7.1%) and 2.10 ml O_2 h^{-1} (4.7%) higher than the mean for the four chambers (42.57 ml O_2 h^{-1} at pre-treatment and 44.36 ml O_2 h^{-1} at mid-treatment), while the RMRs of birds in the fourth metabolic chamber were 3.25 ml O_2 h^{-1} (7.6%) and 4.21 ml O_2 h^{-1} (9.5%) lower, respectively. The range of maximal differences in the RMR of birds measured first (chamber 1) compared with birds measured last (chamber 4) within the same night spanned from –30.0% to +7.7% of RMR associated with chamber 1 at pre-treatment (mean difference: –15% of mean all-chamber RMR). For mid-treatment, this range was –26.7% to +32.6% (mean difference: –7% of mean all-chamber RMR). However, when considering maximal differences in RMR of birds measured twice in the same chamber throughout the experiment (N=7), the range spanned from –22.7% to 30.0% of the first RMR measurement, indicating that the time effect is within the natural individual variation in RMR measurements. Indeed, this time effect proved to be weak since *post-hoc* analysis, using Bonferroni correction for multiple comparisons (Rice, 1989), revealed only a marginally significant difference (P=0.0074, with level of significance corrected to P<0.008) between first and last chambers at pre-treatment but failed to detect any significant differences in RMR between birds held in different chambers at mid-treatment. We nevertheless used a conservative approach and included time in the model when correcting RMR for the effect of body mass. In order to evaluate changes in RMR within individual birds throughout the experiment, we used repeated measures analysis of variance (ANOVA). To control for the effect of body mass in this model we averaged pre- and mid-treatment masses and included it in the model as a covariate (body mass was highly repeatable between pre- and mid-treatment; r^2=0.89, N=32, P<0.0001). However, because we randomized the position of the birds in their metabolic

chambers, we were not able to control for the time effect (i.e. the birds were not consistently put in the same chambers). We do not believe that this introduced a systematic bias. As mentioned earlier, the only detectable time effect was recorded between measurements for chambers 1 and 4 at pre-treatment. However, only seven of 32 birds were measured in these two chambers over the two measurement periods. Out of these seven, only four individuals had their RMR measured in chamber 4 at pre-treatment and in chamber 1 at mid-treatment, which would artificially increase their RMR. Data are reported as means ± S.E.M.

Results

Experiment 1: pattern of yolk precursor plasma levels in response to daily E_2 injections

Administration of estradiol triggered a significant increase in circulating VTG in male and female zebra finches (two-way ANOVA, treatment effect, $F_{4,49}$=5.60, P<0.005), and there was no significant difference in the pattern of response to E_2 treatment between the sexes (sex effect, $F_{1,49}$=2.08, P=0.2; no significant interaction term). Therefore, we pooled the samples to obtain a more accurate picture of the pattern of VTG production (treatment effect $F_{4,50}$=5.31, P<0.005; Fig. 2). In response to daily E_2 injections, plasma VTG increased to reach a peak of 3.2±0.7 μg ml^{-1} on the day following the last injection (day 5 in Fig. 2). This VTG level is higher than previously reported values for breeding female zebra finches at the one-egg stage (1.68 μg ml^{-1}; Williams and Christians, 1997) but well within the normal physiological breeding range (0.54–5.55 μg VTG-Zn ml^{-1} in Williams and Christians, 1997; 0.47–4.26 μg VTG-Zn ml^{-1} in Salvante and Williams, 2002). Bonferroni-corrected, *post-hoc* contrast analysis revealed significant differences between VTG levels at day 2 and day 5 (P<0.005) but showed no significant differences between consecutive days from day 2 to day 5, suggesting a gradual increase in plasma VTG throughout the treatment with no significant changes in the rate of precursor production. We compared these data with VTG levels from an independent group of non-breeding individuals from our colony (N=11). Addition of the non-breeding group did not change the overall analysis (treatment effect, $F_{5,60}$=17.80, P<0.0001) and showed that our treatment clearly triggered a significant increase in VTG plasma levels even after the very first injection (Fig. 2). Indeed, circulating VTG at day 2 was 1518% higher than non-breeding values (independent contrast, P<0.0001). By two days following the last injection (day 6 in Fig. 2), VTG levels decreased to values comparable with those on the first day of blood sampling (day 2; independent contrast, P=0.9), further demonstrating that hepatic VTG production was clearly dependent on E_2 stimulation but also that breakdown of VTG was rapid and comparable in males and females. In fact, 80% of the VTG was already removed from the plasma at day 6 (Fig. 2; day 5 *vs*. day 6 independent contrast; P<0.005). At this rate, only 1.25 days are needed to return plasma VTG to non-breeding levels. Therefore, our treatment induced a high level of hepatic VTG production in parallel with rapid breakdown of the protein. Based on these results, we chose to perform the RMR measurement in experiment 2 after the second injection in order to measure energy expenditure during active hepatic activity.

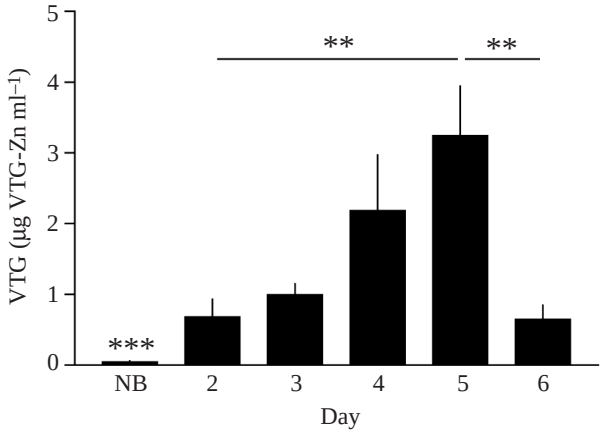

Fig. 2. Pattern of circulating vitellogenin (VTG) measured daily during experiment 1. Horizontal lines indicate significant differences between VTG levels in birds measured on different days. Values are means + S.E.M. 'Day' corresponds to day in Fig. 1A. NB refers to non-breeding values. **P<0.005, ***P<0.0005. Vitellogenin plasma levels for days 2–6 were all significantly different from NB values (P<0.0005 in all cases).

Experiment 2: metabolic costs of yolk precursor production

Based on the results obtained in the first experiment, we measured RMR during the night between the second and third day of E_2 administration (night of day 8; Fig. 1B). At this point, VTG levels were still lower than normal one-egg stage values (Mann–Whitney test, U=137.00, P<0.005; Salvante and Williams, 2002), and it is clear that the liver was actively synthesizing yolk precursors, as plasma VTG increased for at least two more days (Fig. 2).

There was no difference in body mass between E_2-treated and sham birds measured either at pre-treatment or mid-treatment (pre-treatment t-test, t_{30}=0.21, P=0.8; mid-treatment t-test, t_{30}=0.52, P=0.6). Within groups, there was no treatment-related changes in mass in E_2-treated individuals (paired t-test, t_{15}=1.41, P=0.2). However, sham birds lost 2% of their mass between the two RMR measurements (paired t-test, t_{15}=2.52, P<0.05), decreasing from 15.0±0.4 g to 14.6±0.4 g.

As in experiment 1, VTG production significantly increased in E_2-treated individuals (Friedman test, χ^2=27.22, d.f.=2, P<0.001; Fig. 3A), while levels in sham birds remained below the range of VTG for breeding females (Williams and Christians, 1997; Salvante and Williams, 2002) and showed no significant change throughout the experiment (χ^2=1.97, d.f.=2, P=0.3; Fig. 3A). Differences between groups were evident at all periods of the experiment. At pre-treatment, sham birds had significantly higher levels of plasma VTG than E_2-treated birds (Mann–Whitney test, U=53.00, P<0.005; Fig. 3A). However,

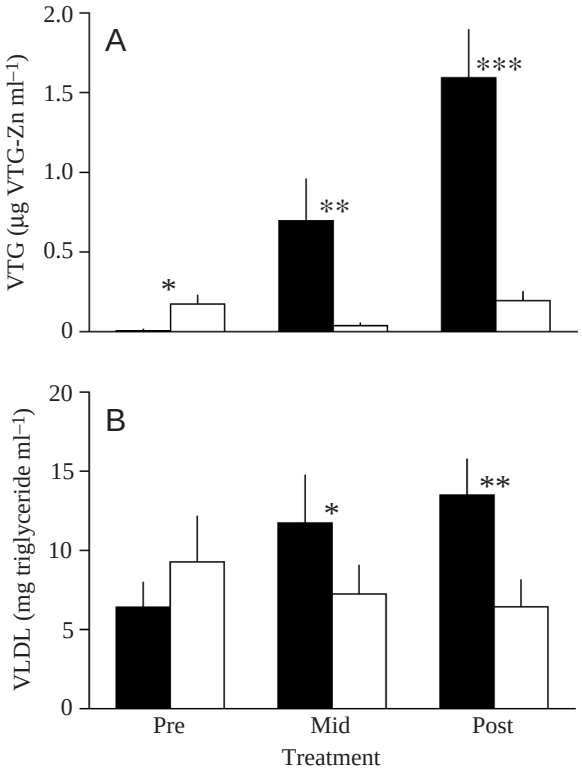

Fig. 3. (A) Plasma vitellogenin (VTG) and (B) very-low-density lipoprotein (VLDL) levels measured at pre-, mid- and post-treatment in estradiol (E_2)-treated (filled bars) and sham groups (open bars). Values are means + S.E.M. Comparisons were made between E_2 and sham treatment groups at pre-, mid- and post-treatment. *P<0.05, **P<0.005, ***P<0.0005. See text for statistics and within-treatment-group differences.

VTG levels in both groups were low and representative of typical non-breeding birds (Williams and Christians, 1997). Therefore, this difference is not biologically relevant. At mid- and post-treatment, E_2 birds exhibited significantly higher levels of VTG than sham individuals (Mann–Whitney test, mid-treatment, U=34.00, P<0.001; post-treatment, U=22.00, P<0.0001; Fig. 3A), with VTG levels averaging 1.59 μg ml^{-1} at post-treatment. This is lower than the reported maximum for the first experiment and it is not clear why the same E_2 dose resulted in different precursor levels. However, plasma VTG levels reported in experiment 2 are much closer to the natural breeding level of 1.68 μg ml^{-1} (Williams and Christians, 1997).

Estradiol administration had a similar effect on the pattern of VLDL production, with plasma levels significantly increasing in E_2-treated individuals (Friedman test, χ^2=11.38, d.f.=2, P<0.005; Fig. 3B). One individual had a surprisingly high VLDL level (106 mg ml^{-1}) at pre-treatment. Taking this individual out of the analysis made the increase in plasma VLDL in the E_2 group more marked (including outlier: 12.64±6.40 mg ml^{-1} at pre-treatment to 15.71±3.05 mg ml^{-1} at post-treatment; excluding outlier: 6.42±1.58 mg ml^{-1} at pre-treatment to 13.51±2.25 mg ml^{-1} at post-treatment) and did not change the overall effect of estradiol administration on plasma

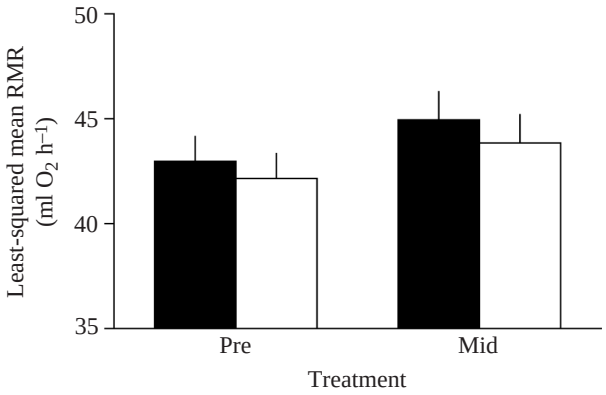

Fig. 4. Comparison of least-squared mean resting metabolic rate (RMR; correcting for the effect of body mass and time) between estradiol (E_2)-treated (filled bars) and sham groups (open bars) at pre- and mid-treatment. Values are means + S.E.M.

VLDL levels (Friedman test, χ^2=13.73, d.f.=2, P<0.005). Conversely, sham individuals showed no change in plasma VLDL (Friedman test, χ^2=4.88, d.f.=2, P=0.08; Fig. 3B). These results translated into no significant difference between groups at pre-treatment (Mann–Whitney test, pre-treatment, U=97.00, P=0.4), 61% higher VLDL levels in E_2-treated birds at mid-treatment (Mann–Whitney test, mid-treatment, U=57.00, P<0.05) and 109% higher VLDL levels at post-treatment (Mann–Whitney test, post-treatment, U=45.00, P<0.005). The maximal VLDL level reported here (post-treatment levels in E_2-treated birds, 13.51 mg ml^{-1}) is 28% lower than previously reported values for female zebra finches at the one-egg stage (18.87 mg ml^{-1}; Williams and Christians, 1997) but still within the natural *wide* range of variation for this lipoprotein (6.4–54.5 mg ml^{-1}; Williams and Christians, 1997).

Pre-treatment RMR did not differ between the experimental groups ($F_{1,26}$=0.21, P=0.7) but was positively related to body mass ($F_{1,26}$=8.76, P<0.01). While mid-treatment RMR was also related to body mass ($F_{1,26}$=10.50, P<0.005), estradiol injections and increased yolk precursor production did not result in higher RMR in E_2-treated birds compared with sham individuals ($F_{1,26}$=0.34, P=0.6; Fig. 4). Similarly, E_2-treated and sham groups did not differ in their RMR response to injections over time (repeated measures ANOVA, treatment × time interaction, $F_{1,29}$=0.01, P=0.9) Indeed, repeated measures ANOVA revealed no significant changes in RMR between pre- and mid-treatment measurements when controlling for body mass ($F_{1,29}$=0.24, P=0.6). Mid-treatment RMR was not correlated with circulating VTG or VLDL levels at mid- or post-treatment (Spearman rank correlation, P>0.3 in all cases) in E_2-treated birds.

Discussion

In this experiment, we demonstrated that male zebra finches respond rapidly (i.e. within 24 h) to exogenous E_2

administration in the form of a gradual increase in plasma VTG and that this response did not differ from that of non-breeding females undergoing the same treatment. This is consistent with previous findings showing that roosters treated with E_2 responded by synthesizing VTG as rapidly as 3–4 h after estradiol injection (Bergink et al., 1974). Our birds also responded to the treatment by increasing VLDL production, which is in accordance with previously reported increases in plasma lipoproteins or liver fat content following exogenous estradiol administration (Yu and Maquardt, 1973a,b; Bergink et al., 1974; Harms et al., 1977; Rosebrough et al., 1982; Williams and Martyniuk, 2000). The rapid decline in VTG after the last injection, however, contradicts previous research where VTG synthesis has been shown to increase linearly for several days (Bergink et al., 1974) or even weeks (Robinson and Gibbins, 1984) after E_2 treatment. This discrepancy is most likely due to the differences in hormone doses administered between studies. In the Bergink et al. (1974) experiment, E_2 was administrated at a dose of 25 mg kg^{-1}, which was 17 times the dose we used. It is clear that the pattern of yolk precursor production reported here, including the rapid breakdown in VTG plasma levels following E_2 administration, is more representative of the 'endogenous response'. This is supported by the fact that VTG levels in our E_2-treated birds remained within the natural range for breeding female zebra finches even after four consecutive days of injection. Our results clearly demonstrated that the livers of our E_2-treated birds were actively synthesizing VTG and VLDL and that active yolk precursor production was maintained as long as E_2 treatment was continued. Similarly, Salvante and Williams (2002) documented a rapid decline in plasma VTG in laying zebra finches nearing clutch completion when endogenous estrogen production is declining (Sockman and Schwabl, 1999).

Comparing our values of RMR with those from the literature suggests that our respirometry technique provided robust estimates of BMR. Resting metabolic rate in pre-treated birds was 42.6 ml O_2 h^{-1}, which is equivalent to 18.8–21.3 kJ day^{-1} for energy substrate going from protein to carbohydrates (Schmidt-Nielsen, 1990; mean respiratory quotient was 0.76, which indicates that the birds could be using energy from mixed sources, which complicates the energetic conversion). This is almost identical to BMR estimates of 19.7 kJ day^{-1} for zebra finches reported by Gavrilov (1997). Our estimate of RMR in pre-treated birds (2.8 ml O_2 h^{-1} when using our mean body mass of 15.1 g) is also very similar to Vleck's published results for incubating zebra finches at 35°C (3.0 ml O_2 h^{-1} in fig. 2 of Vleck, 1981).

However, despite an 80-fold increase in circulating VTG and a 2-fold increase in plasma VLDL in E_2-treated birds, RMR, when measured during active hepatic yolk precursor production, did not increase significantly in E_2-treated individuals compared with pre-treatment measurements or sham individuals. This is consistent with the findings of Vézina and Williams (2003), which showed no relationships between RMR and plasma VTG and VLDL in laying European starlings. There is no other study that we are aware of that has specifically investigated the metabolic cost of producing the yolk precursors.

Our results may be interpreted as a low energy investment in VTG and VLDL synthesis. However, this would be surprising given the marked charges in protein and lipid metabolism associated with rapid yolk formation (Gruber, 1972; Griffin and Hermier, 1988). We suggest that there might be an alternative explanation. There is accumulating evidence that birds can adjust their energy consumption in a compensatory manner when challenged by multiple, and perhaps competing, high-energy demands. For example, a decrease in locomotor activity has been suggested to compensate for the cost of molting (Austin and Fredrickson, 1987), for the reduced energy availability in fasting birds (Cherel et al., 1988) and for the cost of egg formation (Houston et al., 1995; Williams and Ternan, 1999). Similarly, the heat generated by feeding (Masman et al., 1989) or foraging activity (Webster and Weathers, 1990; Bruinzeel and Piersma, 1998) has been shown to partially compensate for thermoregulatory costs. It is clear that changes occurring in physiological systems and organs within an individual may also result in compensatory effects leading to energy reallocation with no net increase in overall energy consumption. For example, Geluso and Hayes (1999) measured BMR and organ composition of starlings under high- and low-quality diets. They found significant differences in the mass of the gastrointestinal tract, gizzard, liver and breast muscle but no differences in BMR, indicating that upregulation of certain organs or systems may be coincident with downregulation of other systems or organs in order to maintain a constant maintenance energy cost. The question of whether the higher hepatic activity involved in VTG and VLDL production results in the downregulation of other physiological systems remains to be resolved. Clearly, studies investigating energy expenditure at the organ level are needed to elucidate this hypothesis. We know from previous work in our laboratory that liver structural size does not systematically increase in association with high levels of endogenous plasma yolk precursor production in European starlings and zebra finches (Christians and Williams, 1999a; Williams and Martyniuk, 2000; Vézina and Williams, 2003) or even in response to administration of exogenous estradiol (Christians and Williams, 1999b; Williams and Martyniuk, 2000). If energy reallocation does take place during precursor production, the metabolic intensity of the liver (i.e. energy consumption per unit mass) may still be high during VTG and VLDL production even though mass-corrected RMR was independent of plasma yolk precursor levels in our study. A possible mechanism for this could be that the regulation of other physiological systems is also triggered by a rise in plasma E_2. For example, immune function, which has been shown to have a measurable metabolic cost (Demas et al., 1997; Raberg et al., 2001; Martin et al., 2002), can be inhibited by certain doses of estradiol in chickens (al-Afalek and Homeida, 1998; Landsman et al., 2001). Therefore, a reduction in the activity of the immune system could potentially lead to energy savings that can be reallocated to other functions.

This experiment was specifically designed to measure the potential costs of yolk precursor production. However, the influence of other aspects of egg formation on overall energy expenditure should also be examined. For example, egg yolk mass is related to the rate of yolk precursor uptake at the ovary and is potentially limited by the number of VTG/VLDL receptors and their rate of recycling (Christians and Williams, 2001). Therefore, the very active process of rapid yolk development may also result in substantial energy investment. These aspects of egg formation cannot be assessed using males or even non-breeding females due to the lack of developing ovarian follicles. Thus, more investigation is needed to explain the remaining variation in elevated laying RMR (Nilsson and Raberg, 2001; Vézina and Williams, 2002) not accounted for by the maintenance and activity costs of the oviduct (Vézina and Williams, 2003).

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References

Al-Afalek, A. I. and Homeida, A. M. (1998). Effects of low doses of oestradiol, testosterone and dihydrotestosterone on the immune response of broiler chicks. *Immunopharmacol. Immunotoxicol.* **20**, 315-327.

Austin, J. E. and Fredrickson, L. H. (1987). Body and organ mass and body composition of postbreeding female lesser scaup. *Auk* **104**, 694-699.

Bergink, E. W., Wallace, R. A., Van De Berg, J. A., Bos, E. S., Gruber, M. and Geert, A. B. (1974). Estrogen-induced synthesis of yolk proteins in roosters. *Am. Zool.* **14**, 1177-1193.

Bernardi, G. and Cook, W. H. (1960). An electrophoretic and ultracentrifugal study on the proteins of the high density fraction of egg yolk. *Biochimica Biophysica Acta* **44**, 86-96.

Blem, C. R. (2000). Energy balance. In *Sturkie's Avian Physiology* (ed. G. C. Whittow), pp. 327-341. San Diego: Academic Press.

Bruinzeel, L. W. and Piersma, T. (1998). Cost reduction in the cold: heat generated by terrestrial locomotion substitutes for thermoregulation costs in knot *Calidris canutus*. *Ibis* **14**, 323-328.

Challenger, W. O., Williams, T. D., Christians, J. K. and Vézina, F. (2001). Follicular development and plasma yolk precursor dynamics through the laying cycle in the European starling (*Sturnus vulgaris*). *Physiol. Biochem. Zool*. **74**, 356-365.

Chappell, M. A., Bech, C. and Buttemer, W. A. (1999). The relationship of central and peripheral organ masses to aerobic performance variation in house sparrows. *J. Exp. Biol.* **202**, 2269-2279.

Cherel, Y., Robin, J-P. and Le Maho, Y. (1988). Physiology and biochemistry of long-term fasting in birds. *Can. J. Zool.* **66**, 159-166.

Christians, J. K. and Williams, T. D. (1999a). Organ mass dynamics in relation to yolk precursor production and egg formation in European starlings *Sturnus vulgaris*. *Physiol. Biochem. Zool.* **72**, 455-461.

Christians, J. K. and Williams, T. D. (1999b). Effects of exogenous 17β-estradiol on the reproductive physiology and reproductive performance of European starlings (*Sturnus vulgaris*). *J. Exp. Biol.* **202**, 2679-2685.

Christians, J. K. and Williams, T. D. (2001). Interindividual variation in yolk mass and the rate of growth of ovarian follicles in the zebra finch (*Taeniopygia guttata*). *J. Comp. Physiol. B* **171**, 255-261.

Deeley, R. G., Mullinix, K. P., Wetekam, W., Kronenberg, H. M., Meyers, M., Eldridge, J. D. and Golberger, R. F. (1975). Vitellogenin synthesis in the avian liver. *J. Biol. Chem.* **250**, 9060-9066.

Demas, G. E., Chefer, V., Talan, M. I. and Nelson, R. J. (1997). Metabolic costs of mounting an antigen-stimulated immune response in adult and aged C57BL/6J mice. *Am. J. Physiol.* **273**, R1631-R1637.

Follett, B. K. and Redshaw, M. R. (1974). The physiology of vitellogenesis. In *Physiology of the Amphibia*, vol. 2 (ed. B. Lofts), pp. 219-308. New York: Academic Press.

Gavrilov, V. M. (1997). *Energetics and Avian Behaviour*. Amsterdam, The Netherlands: Harwood Academic.

Geluso, K. and Hayes, J. P. (1999). Effects of dietary quality on basal metabolic rate and internal morphology of European starlings (*Sturnus vulgaris*). *Physiol. Biochem. Zool.* **72**, 189-197.

Griffin, H. and Hermier, D. (1988). Plasma lipoprotein metabolism and fattening in poultry. In *Leanness in Domestic Birds* (ed. B. Leclercq and C. C. Whitehead), pp. 175-202. London: Butterworths.

Gruber, M. (1972). Hormonal control of yolk protein synthesis. In *Egg Formation and Production.* (ed B. M. Freeman and P. E. Lake), pp. 23-32. Edinburgh: British Poultry Science.

Harms, R. H., Roland, D. A. and Simpson, C. F. (1977). Experimentally induced "fatty liver syndrome" condition in laying hens. *Poult. Sci.* **56**, 517-520.

Hayes, J. P., Speakman, J. R. and Racey, P. A. (1992). Sampling bias in respirometry. *Physiol. Zool.* **65**, 604-619.

Hermier, D., Forgez, P., Williams, J. and Chapman, M. J. (1989). Alteration in plasma lipoproteins and apolipoproteins associated with estrogen-induced hyperlipidemia in the laying hen. *Eur. J. Biochem.* **184**, 109-118.

Houston, D. C., Donnan, D. and Jones, P. J. (1995). The source of the nutrients required for egg production in zebra finches *Poephila guttata*. *J. Zool. Lond.* **235**, 469-483.

Landsman, T., Leitner, G., Robinzon, T. B. and Heller, E. D. (2001). Effect of gonadal steroids on proliferative responses and subset alterations in cultured chicken lymphocytes. *Poult. Sci.* **80**, 1329-1338.

Martin, L. B., II, Scheuerlein, A. and Wikelski, M. (2002). Immune activity elevates energy expenditure of house sparrows: a link between direct and indirect costs? *Proc. R. Soc. Lond. B* **270**, 153-158.

Masman, D., Daan, S. and Dietz, M. (1989). Heat increment of feeding in the kestrel, *Falco tinnunculus*, and its natural seasonal variation. In *Physiology of Cold Adaptation in Birds* (ed. C. Bech and R. E. Reinertsen), pp. 83-94. New York: Plenum Press.

Meijer, T., Rozman, J., Schulte, M. and Stach-Dreesmann, C. (1996). New findings in body mass regulation in zebra finches (*Taeniopygia guttata*) in response to photoperiod and temperature. *J. Zool. Lond.* **240**, 717-734.

Mitchell, M. A. and Carlisle, A. J. (1991). Plasma zinc as an index of vitellogenin production and reproductive status in the domestic fowl. *Comp. Biochem. Physiol. A* **100**, 719-724.

Monaghan, P. and Nager, R. G. (1997). Why don't birds lay more eggs? *Trends Ecol. Evol.* **12**, 270-274.

Nilsson, J.-A. and Raberg, L. (2001). The resting metabolic cost of egg laying and nestling feeding in great tits. *Oecologia* **128**, 187-192.

Raberg, L., Vestberg, M., Hasselquist, D., Holmdahl, R., Svensson, E. and Nilsson, J.-A. (2001). Basal metabolic rate and the evolution of the adaptive immune system. *Proc. R. Soc. Lond. B* **269**, 817-821.

Rice, W. R. (1989). Analyzing tables of statistical tests. *Evolution* **43**, 223-225.

Robinson, G. A. and Gibbins, A. M. V. (1984). Induction of vitellogenesis in Japanese quail as a sensitive indicator of the estrogen-mimetic effects of a variety of environmental contaminants. *Poult. Sci.* **63**, 1529-1536.

Rosebrough, R. W., McMurtry, J. P. and Steele, N. C. (1982). Effect of estradiol on the lipid metabolism of young turkey hens. *Nutr. Rep. Int.* **26**, 373-376.

Salvante, K. G. and Williams, T. D. (2002). Vitellogenin dynamics during egg-laying: daily variation, repeatability and relationship with egg size. *J. Avian Biol.* **33**, 391-398.

Scanes, C. G. (2000). Introduction to endocrinology: In *Sturkie's Avian Physiology* (ed. G. C. Whittow), pp. 437-460. San Diego: Academic Press.

Schmidt-Nielsen, K. (1990). *Animal Physiology, Adaptation and Environment.* Cambridge: Cambridge University Press.

Sockman, K. W. and Schwabl, H. (1999). Daily estradiol and progesterone levels relative to laying and onset of incubation in canaries. *Gen. Comp. Endocrinol.* **114**, 257-268.

Stearns, S. C. (1992). *The Evolution of Life Histories.* Oxford: Oxford University Press.

Stifani, S., George, R. and Schneider, W. J. (1988). Solubilization and characterization of the chicken oocyte vitellogenin receptor. *Biochem. J.* **250**, 4647-4675.

Vézina, F. and Williams, T. D. (2002). Metabolic costs of egg production in the European starling (*Sturnus vulgaris*). *Physiol. Biochem. Zool.* **75**, 377-385.

Vézina, F. and Williams, T. D. (2003). Plasticity in body composition in breeding birds: what drives the metabolic costs of egg production? *Physiol. Biochem. Zool.* In press.

Vleck, C. M. (1981). Energetic cost of incubation in the zebra finch. *Condor* **83**, 229-237.

Wallace, R. A. (1985). Vitellogenesis and oocyte growth in nonmammalian vertebrates. In *Developmental Biology*, vol. 1 Oogenesis. (ed. L. W. Brower), pp. 127-177. New York: Plenum Press.

Walzem, R. L. (1996). Lipoproteins and the laying hen: form follows function. *Poult. Avian Biol. Rev*. **7**, 31-64.

Walzem, R. L., Davis, P. A. and Hansen, R. J. (1994). Overfeeding increases very low density lipoprotein diameter and causes the appearance of a unique lipoprotein particle in association with failed yolk deposition. *J. Lipid Res.* **35**, 1354-1366.

Walzem, R. L., Hansen, R. J., Williams, D. L. and Hamilton, R. L. (1999). Estrogen induction of VLDLy assembly in egg-laying hens. *J. Nutr*. **129**, 467S-472S.

Webster, M. D. and Weathers, W. W. (1990). Heat production as a by-product of foraging activity contributes to thermoregulation by verdins, *Auriparus flaviceps*. *Physiol. Zool*. **63**, 777-794.

Williams, T. D. (1998). Avian reproduction, overview. In *Encyclopedia of Reproduction*, vol. 1. (ed. E. Knobil and J. D. Neil), pp. 325-336. New York: Academic Press.

Williams, T. D. and Christians, J. K. (1997). Female reproductive effort and individual variation: neglected topics in environmental endocrinology? In *Proc. XIII Int. Congr. Comp. Endocrinol.* (ed. S. Kawashima and S. Kikuyama), pp. 1669-1675. Rome: Monduzzi Editore.

Williams, T. D. and Martyniuk, C. J. (2000). Tissue mass dynamics during egg-production in female zebra finches (*Taeniopygia guttata*): dietary and hormonal manipulations. *J. Avian Biol.* **31**, 87-95.

Williams, T. D. and Ternan, S. P. (1999). Food intake, locomotor activity, and egg laying in zebra finches: contributions to reproductive energy demand? *Physiol. Zool.* **72**, 19-27.

Yu, J. Y.-L. and Maquardt, R. R. (1973a). Effects of estradiol and testosterone on the immature female chicken (*Gallus domesticus*)-I. Quantitative changes in nucleic acids, proteins and lipids in the liver. *Comp. Biochem. Physiol. B* **46**, 749-757.

Yu, J. Y.-L. and Maquardt, R. R. (1973b). Effects of estradiol and testosterone on the immature female chicken (Gallus domesticus)-II. Immunodiffusional patterns of yolk lipoproteins in the liver and plasma. *Comp. Biochem. Physiol. B* **46**, 759-767.

Zar, J. H. (1996). *Biostatistical Analysis.* London: Prentice Hall.