Egg mass shows large intraspecific variation in birds and yet the mechanisms underlying this variation remain unknown. We hypothesized that estradiol would play a central role in determining egg mass, since this hormone stimulates the production of yolk precursors (vitellogenin and very-low density lipoprotein, VLDL) by the liver, and of albumen by the oviduct. We gave European starlings (Sturnus vulgaris) silastic implants containing estradiol prior to egg formation, which we predicted would increase egg mass. As expected, exogenous estradiol stimulated a marked (49 %) increase in plasma vitellogenin levels at the beginning of laying. At clutch completion, plasma VLDL levels and oviduct mass were also elevated in estradiol-treated females compared with controls. However, estradiol had no effect on fresh egg mass or clutch size. Estradiol treatment actually decreased the mass of yolk protein and lipid, perhaps by decreasing the rate of uptake of yolk precursors at the ovary. The failure of estradiol to increase egg mass indicates that this phenotype may be regulated at higher levels of organization (e.g. negative feedback, uptake of yolk precursors) than those studied in this experiment. Despite elevating yolk precursor levels, treatment with estradiol had no effect on the mass of the liver or endogenous stores of protein and lipid at clutch completion.

Key words: egg production, yolk, oocyte growth, vitellogenin, intraspecific variation, starling, Sturnus vulgaris.

Summary

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Key words: egg production, yolk, oocyte growth, vitellogenin, intraspecific variation, starling, Sturnus vulgaris.

Introduction

Reproduction necessitates the allocation of resources among competing demands (Stearns, 1992). Trade-offs may occur within reproductive bouts (e.g. between propagule size and number; but see Bernardo, 1996) and between breeding attempts, through effects of current reproductive effort on survival and future fecundity (Stearns, 1992). Reproductive trade-offs have received a great deal of attention in ecological and evolutionary studies, and yet little is known about their physiological basis (Sheldon and Verhulst, 1996). For example, recent studies of birds have suggested that increased expenditure during egg production may adversely affect the female’s ability to rear offspring (Heaney and Monaghan, 1995; Monaghan and Nager, 1997; Monaghan et al., 1998). The mechanism(s) underlying this cost are not understood, although parasitism may be involved (Oppliger et al., 1996). Furthermore, while these studies have examined the costs of laying more eggs, the costs of laying larger eggs remain unknown. Currently such costs cannot be examined experimentally because of the lack of a technique to manipulate egg size without affecting the female’s nutritional state (e.g. Selman and Houston, 1996; Williams, 1996b), energetic expenditure on other activities (e.g. thermoregulation; Nager and van Noordwijk, 1992) or clutch size (e.g. Sinervo and Licht, 1991). Development of such a technique is hindered by our lack of understanding of the mechanism(s) that determine egg quality in birds (Williams, 1996a).

Estradiol would be expected to play a central role in the determination of egg mass and quality. This hormone stimulates the avian liver to produce the yolk precursors (vitellogenin and very-low density lipoprotein, VLDL), the primary sources of yolk protein and lipid, respectively (Wallace, 1985). The production of these yolk precursors occurs not only in females forming eggs, but also in males and immature females treated with estradiol (Yu and Marquardt, 1973; Wiskocil et al., 1980; Wallace, 1985). The synthesis and secretion of egg albumen by the oviduct is also, in part, under estrogenic control (Brant and Nalbandov, 1956; Yu et al., 1971). Although a great deal of work has examined the effects of exogenous estradiol on males and immature birds (e.g. Yu et al., 1971; Yu and Marquardt, 1973; Rosebrough et al., 1982; Wallace, 1985), few studies have attempted to use this hormone to manipulate the physiology or egg-production performance of breeding females (but see Williams, 1999).
We investigated the role of estradiol in the determination of primary reproductive effort in a free-living, nest-box population of European starlings (*Sturnus vulgaris*). Females were treated with estradiol prior to and during egg formation, when circulating levels of estradiol are naturally high (Dawson, 1983). Thus, we sought to augment the plasma estradiol concentration in an additive manner, but still within the range of natural variation, to cause physiological rather than pharmacological effects. The effects of exogenous estradiol on individual breeding females were assessed by measuring reproductive effort before and after estradiol treatment. We hypothesized that treatment with estradiol would increase egg quality via two mechanisms: (1) increased vitellogenin and VLDL production by the liver, and hence larger yolks, and (2) increased albumen production by the oviduct. Furthermore, we predicted that (1) the increased production of yolk precursors and albumen would lead to hypertrophy of the liver and oviduct, respectively; and (2) the increased investment in eggs would result in the depletion of endogenous stores of protein or specific amino acids (e.g. those located in the flight muscles; Houston et al., 1995), and lipid. Treated females were studied on the night after laying the first egg of their clutch, since various models (e.g. Ojane, 1983; Houston et al., 1995) indicate that this is close to the time of maximal nutritional and energetic investment in eggs, i.e. the peak of egg production effort. Females were also examined at clutch completion, at which point the cumulative costs of egg production would have incurred.

**Materials and methods**

**General**

Field work was performed during the breeding seasons of 1996 and 1997 at the Pacific Agri-food Research Centre (PARC) in Agassiz, British Columbia (49° 14' N, 121° 46' W), using a nest-box population of European starlings *Sturnus vulgaris* L. The experimental protocol followed the guidelines of the Canadian Committee on Animal Care (Simon Fraser University Animal Care Committee Project Number 442B; PARC ACC Experiment Number 9702).

**Estradiol treatment**

Females were captured in the nest box after laying a first, unmanipulated clutch, generally 2 or 3 (in a few cases, 4 or 5) days after the last egg was laid. Birds were anesthetized (using a mixture of ketamine and xylazine at doses of 20 mg kg\(^{-1}\) and 4 mg kg\(^{-1}\), respectively), and given two subcutaneous silastic implants (length 15 mm, 1.5 mm i.d., 2.0 mm o.d.; Corning) containing 17β-estradiol (Sigma; \(N=16\) in 1996 and \(N=22\) in 1997). An equal number of control birds received sham (empty) implants. Preliminary analyses of the data from the first season suggested that the effects of the estradiol implants were subtle. Therefore, in the second year of the study, females also received a single intramuscular injection of either estradiol at 1 μg g\(^{-1}\) in 40 μl of 1,2-propanediol (Sigma; treatment females) or vehicle only (sham females). Birds were weighed (to within ±1 g), banded with an aluminum US Fish and Wildlife Service band to permit later identification, and returned to their nest box as they recovered from the anesthetic. Their first clutch was removed to induce renesting.

Following the experimental manipulation, we continued to check nest boxes to relocate renesting experimental females, and to determine laying date, egg mass and clutch size for replacement clutches. Females located the night after laying the first egg of their replacement clutch (1-egg stage) were collected for body composition analysis (treatment \(N=7\) in 1996 and \(N=4\) in 1997; sham \(N=4\) in 1996 and \(N=3\) in 1997). Most females were collected between 21:00 and 1:00 h; the two females not collected within this time period were excluded from analyses of yolk precursors and body composition.

In 1996, females that were not located until the completion of the replacement clutch were weighed (to within ±1 g) 2 or 3 days after clutch completion (generally between 21:00 and 1:30 h), and then released (treatment \(N=5\); sham \(N=6\)). In 1997, females recaptured at clutch completion were collected for body composition analysis (between 23:00 and 1:30 h; treatment \(N=6\); sham \(N=6\)). All collected birds were killed by exsanguination under anesthesia (as above), usually within 15 min of capture, and frozen until further analysis.

**Body composition**

The liver, oviduct (the oviducal egg was removed before weighing), ovary and the *pectoralis* and *supracoracoideus* muscles (hereafter referred to as flight muscles) were dissected from the carcass. The rest of the carcass was autoclaved for 9 hours, homogenized and a subsample was obtained for subsequent analyses.

The lean dry masses of the organs (except the ovary) and of the carcass were obtained by drying to constant mass (either in a drying oven at 60 °C or in a freeze drier) followed by Soxhlet extraction for 8 h, with petroleum ether as the solvent (Dobush et al., 1985). Total body fat was calculated from the difference between the dry mass and the dry lean mass of the organs and carcass. In the case of the liver, dry lean glycogen-free mass is reported. Liver glycogen was determined using a modification of the method described by Keppler and Decker (1984), using a subsample of liver (approximately 40 mg) that had been frozen in liquid nitrogen immediately after collection. Further details on the measurement of liver glycogen are provided by Christians and Williams (1999). Wet masses are reported for the ovary.

**Measurement of yolk precursors**

Plasma vitellogenin levels were measured as vitellogenic zinc (Mitchell and Carlisle, 1991; Zinc: Wako Chemicals). Plasma triglyceride was used as an index of VLDL level (Mitchell and Carlisle, 1991; Triglyceride E: Wako Chemicals). Validation of these methods for passerines has been described by Williams and Christians (1997) and Williams and Martyniuk (1999). The inter-assay coefficients of variation for the vitellogenic zinc and triglyceride assays
Effects of estradiol on egg production

Egg mass and composition
Eggs were either weighed (to within ±0.01 g) within 24 h of laying or were measured (length and breadth, to within ±0.01 mm) at clutch completion. Fresh egg mass was calculated from egg dimensions using an empirical formula derived from 175 eggs measured within 12 h of laying ($r^2=0.98$). If no new eggs were found on 2 consecutive days, a clutch was assumed to be complete.

The macronutrient composition of the first egg of the replacement clutch was determined for females collected at the 1-egg stage. Within 24 h of laying, eggs were weighed, boiled for 10 min and then frozen. Eggs were later separated into shell, albumen and yolk. All components were dried and lipids were extracted from the yolks, as described above. Dry albumen and lean dry yolk are approximately 88% protein (Burley and Vadehra, 1989), and were used as measures of albumen and yolk protein, respectively.

Statistics
All statistical analyses were carried out using SAS (SAS Institute, 1988). Preliminary analyses including year and year by treatment interaction terms as covariates in a general linear model (proc GLM; SAS Institute, 1988) indicated that data could be pooled between years. Parametric tests were performed for most variables (general linear model wherever $F$-values are provided; proc GLM, SAS Institute, 1988), but rank-order statistics were used to analyse clutch size, laying date and renesting interval (Wilcoxon two-sample test wherever Z values are provided; proc NPAR1WAY, SAS Institute, 1988), except where noted. Within females, egg mass was highly repeatable among pre- and post-treatment clutches. Therefore, when comparing egg mass between treatments, pre-treatment egg mass was used as a covariate in a general linear model (proc GLM; SAS Institute, 1988) to remove variation due to differences between females. Similarly, in the analysis of post-treatment clutch size, pre-treatment clutch size and renesting interval were used as covariates. In analyses of variables which were correlated with dry lean non-reproductive body mass (i.e. mass of ovary and oviduct not included), the latter term was used as a covariate in a general linear model (proc GLM; SAS Institute, 1988) to statistically control for the effect of body mass. In the case of tissues, body mass – the mass of the tissue was used as the covariate to avoid the effects of part-whole correlation (Christians, 1999). Values are presented as least-squares means ± s.e.m. (SAS Institute, 1988).

93% of females that renested (38/41) laid the first egg of the replacement clutch within 16 days of treatment; most (30/41) renested within 10 days. Unusually long renesting intervals were observed in one estradiol-treated female (20 days) and two sham birds (29 and 44 days). Because these three birds had a much longer period of time in which to recover from treatment and to form a replacement clutch, they were excluded from all analyses except those of renesting interval. One sham female with an unusually long renesting interval (16 days) was collected after one egg had been found in her nest but, based on the number of post-ovulatory follicles in the ovary, had actually laid at least two eggs. Since this bird was not at the 1-egg stage it was also excluded from analyses. Including these four birds in analyses did not qualitatively affect the results.

Results

Pre-treatment reproductive parameters and body mass
To confirm that females had been assigned to treatments at random, we compared the pre-treatment characteristics of females treated with estradiol with those of sham females. Only females that laid a replacement clutch were included in these analyses. No significant differences between treatments were observed in pre-treatment body mass at clutch completion ($F_{1,25}=0.43, P>0.2$), nor egg mass ($F_{1,35}=2.16, P>0.1$), clutch size (Wilcoxon $Z=-0.23, P>0.2$) or laying date ($Z=-0.45, P>0.2$).

Not all experimental females were recaptured, and therefore the birds we observed laying a replacement clutch may not have been a random sample of treated birds (e.g. if only high quality birds produced a replacement clutch). We therefore compared pre-treatment characteristics between females that did and did not renest in our nest boxes. The differences in pretreatment reproductive output between renesting and non-renesting females were not dependent upon treatment (renesting by treatment interaction terms: mean egg mass, $F_{1,68}=0.49, P>0.2$; clutch size, $F_{1,68}=1.33, P>0.2$; laying date, $F_{1,67}=0.04, P>0.2$) and were not significant (mean egg mass, $F_{1,70}=0.16, P>0.2$; clutch size, $Z=1.03, P>0.2$; laying date, $Z=-0.02, P>0.2$). Thus, our experimental protocol did not appear to select for birds with higher reproductive output. However, in the case of body mass, the renesting by treatment interaction term was marginally nonsignificant ($F_{1,48}=2.72, P=0.11$), suggesting that the difference between renesting and non-renesting females may have differed between treatments. Among sham-treated birds, birds that renested were heavier (84.6±1.0 g) than those that did not (81.4±1.0 g; $F_{1,21}=4.52, P<0.05$). Among estradiol-treated birds, however, the mass of renesting birds (83.6±0.8 g) did not differ from that of non-renesting birds (83.8±0.9 g; $F_{1,26}=0.04, P>0.2$). It should be noted that birds that did not renest in the nest-boxes may have renested elsewhere, since many other nesting sites were available.

Effect of exogenous estradiol on reproductive output in post-treatment clutches
Treatment with exogenous estradiol did not affect the probability of renesting ($\chi^2=0.88, P>0.2$; Table 1). Renesting interval (number of days between removal of first clutch and initiation of second clutch), did not differ between treatments, regardless of whether birds with renesting intervals greater


than 15 days were included ($Z=1.26$, $P>0.2$) or excluded from analyses ($Z=0.73$, $P>0.2$; Table 1). Similarly, estradiol and sham birds did not differ in clutch size ($F_{1,17}=0.23$, $P>0.2$; controlling for pre-treatment clutch size and renesting interval; Table 1). Among birds that completed their replacement clutch, post-treatment mean egg mass did not differ between treatments ($F_{1,18}=0.11$, $P>0.2$; Table 1). Similarly, among all renesting birds, there was no difference between estradiol and sham females in the mass of the first egg of the replacement clutch (controlling for the mass of the first egg of the pre-treatment clutch, $F_{1,32}=2.25$, $P>0.1$). The masses of yolk lipid and yolk protein were lighter in the first eggs of estradiol-treated females than in those of controls (yolk lipid: $F_{1,13}=6.54$; yolk protein: $F_{1,13}=6.87$; $P=0.02$ in both cases; Table 1). There was no detectable effect of treatment on the mass of albumen protein ($F_{1,13}=0.11$, $P>0.2$; Table 1) or dry shell ($F_{1,14}=0.05$, $P>0.2$; Table 1).

**Effect of exogenous estradiol on the plasma levels of the yolk precursors**

Among females collected at the 1-egg stage, plasma vitellogenin levels were 49% higher in estradiol-treated females than in controls ($F_{1,12}=8.12$, $P<0.05$; Table 2). Plasma vitellogenin ranged from 2.67 μg vitellogenic zinc ml$^{-1}$ to 5.78 μg ml$^{-1}$ in estradiol-treated birds and 2.39 μg ml$^{-1}$ to 3.33 μg ml$^{-1}$ in sham birds. Plasma VLDL levels at the 1-egg stage were also higher in estradiol-treated females, but this difference was not significant ($F_{1,12}=1.37$, $P>0.2$). Plasma VLDL ranged from 19.4 mg ml$^{-1}$ (triglyceride) to 47.0 mg ml$^{-1}$ in estradiol-treated birds and 14.8 mg ml$^{-1}$ to 27.1 mg ml$^{-1}$ in sham birds. At clutch completion, estradiol-treated females had higher levels of both plasma vitellogenin ($F_{1,8}=9.10$, $P<0.05$) and plasma VLDL ($F_{1,8}=6.05$, $P<0.05$).

**Effect of exogenous estradiol on body composition**

Estradiol treatment had no detectable effect on the mass of the reproductive tissue at the 1-egg stage (wet mass of the ovary: $F_{1,11}=0.01$, $P>0.2$; lean dry mass of the oviduct: $F_{1,12}=1.19$, $P>0.2$; Table 2). At clutch completion, however, estradiol-treated females had lighter ovaries ($F_{1,8}=13.76$, $P<0.01$), and heavier oviducts ($F_{1,8}=19.29$, $P<0.01$) than sham birds (Table 2). The lean dry glycogen-free mass of the liver, another organ intimately involved in reproduction, did not change.

**Table 1. Comparison of primary reproductive output in the post-treatment clutch of estradiol- and sham-treated female European starlings**

<table>
<thead>
<tr>
<th>Variable</th>
<th>Estradiol</th>
<th>Sham</th>
</tr>
</thead>
<tbody>
<tr>
<td>Renesting probability</td>
<td>57% (21/37)</td>
<td>46% (16/35)</td>
</tr>
<tr>
<td>Renesting interval (days)</td>
<td>8.5±0.5 (21)</td>
<td>9.1±0.5 (16)</td>
</tr>
<tr>
<td>Clutch size</td>
<td>5.6±0.2 (10)</td>
<td>5.7±0.2 (11)</td>
</tr>
<tr>
<td>Mean egg mass$^b$ (g)</td>
<td>7.29±0.08 (10)</td>
<td>7.25±0.07 (11)</td>
</tr>
<tr>
<td>Mass of first egg$^b$ (g)</td>
<td>6.92±0.10 (21)</td>
<td>7.16±0.12 (14)</td>
</tr>
<tr>
<td>Yolk lipid of first egg (mg)</td>
<td>265±8 (11)</td>
<td>304±13 (4)*</td>
</tr>
<tr>
<td>Yolk protein of first egg (mg)</td>
<td>180±5 (11)</td>
<td>203±8 (4)*</td>
</tr>
<tr>
<td>Albumen protein of first egg (mg)</td>
<td>528±15 (11)</td>
<td>518±25 (4)</td>
</tr>
<tr>
<td>Dry shell of first egg (mg)</td>
<td>422±11 (11)</td>
<td>426±16 (5)</td>
</tr>
</tbody>
</table>

Values are least-squares means ± S.E.M. Sample sizes are in parentheses.

$^a$Controlling for pre-treatment clutch size and renesting interval.

$^b$Controlling for pre-treatment value.

$^*P<0.05$.

**Table 2. Comparison of physiological variables of estradiol- and sham-treated female European starlings at the 1-egg stage and at the completion of the post-treatment clutch**

<table>
<thead>
<tr>
<th>Variable</th>
<th>1-egg stage</th>
<th>Clutch completion</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Estradiol</td>
<td>(N=9)</td>
</tr>
<tr>
<td>Plasma vitellogenin (μg ml$^{-1}$ vitellogenic zinc)</td>
<td>4.35±0.30</td>
<td>2.92±0.40*</td>
</tr>
<tr>
<td>Plasma VLDL (mg ml$^{-1}$ triglyceride)</td>
<td>27.3±2.6</td>
<td>22.2±3.5</td>
</tr>
<tr>
<td>Wet ovary (mg)</td>
<td>2160±124</td>
<td>2190±183</td>
</tr>
<tr>
<td>Lean dry oviduct (mg)</td>
<td>857±31</td>
<td>913±41</td>
</tr>
<tr>
<td>Lean dry glycogen-free liver mass (mg)</td>
<td>924±25</td>
<td>964±34</td>
</tr>
<tr>
<td>Lean dry flight muscle (g)</td>
<td>4.22±0.11</td>
<td>4.79±0.14*</td>
</tr>
<tr>
<td>Total body lipid (g)</td>
<td>2.84±0.25</td>
<td>2.87±0.33</td>
</tr>
<tr>
<td>Fresh body mass (g)</td>
<td>97.8±0.9</td>
<td>100.9±1.2</td>
</tr>
</tbody>
</table>

Values are least-squares means ± S.E.M.

$^*P<0.05$, comparing between treatments within each stage.
differ between treatments at the 1-egg stage \((F_{1,12}=0.90, P>0.2)\) or at clutch completion \((F_{1,7}=0.00, P>0.2); \text{ Table 2}\).

A potential endogenous protein store, flight muscle, was lighter in estradiol-treated females at the 1-egg stage \((F_{1,12}=10.41, P<0.01)\), but not at clutch completion \((F_{1,7}=0.05, P>0.2); \text{ Table 2}\). Similarly, fresh body mass was lighter in estradiol-treated females at the 1-egg stage, although this difference was marginally nonsignificant (controlling for pre-treatment body mass, \(F_{1,11}=3.67, P=0.08; \text{ Table 2}\)). No treatment effect on body mass at clutch completion was observed (controlling for pre-treatment body mass, \(F_{1,18}=2.57, P>0.1; \text{ Table 2}\)). Total body lipid did not differ between treatments at the 1-egg stage \((F_{1,12}=0.00, P>0.1)\) or at clutch completion \((F_{1,8}=0.04, P>0.2); \text{ Table 2}\).

**Discussion**

The primary goal of this study was to induce birds to produce larger eggs so that we could elucidate the mechanisms which determine egg mass and the costs of increased investment in eggs. Clearly, a given species of bird is capable of producing eggs of a wide range of sizes (e.g. Cooke et al., 1995; Williams, 1996a). Because of the central role of estradiol in egg production, we gave females subcutaneous implants which are known to be effective in elevating estradiol levels during breeding (e.g. Wingfield et al., 1989). As predicted, estradiol treatment elevated plasma vitellogenin levels at the 1-egg stage by 49%, and also increased plasma VLDL levels and oviduct mass at clutch completion. We hypothesized that these effects would stimulate an increase in egg mass and lead to generally higher reproductive effort among estradiol-treated females. However, exogenous estradiol had no detectable effect on egg mass, clutch size, renesting interval or probability of renesting. In contrast with our prediction, estradiol treatment decreased the mass of yolk protein and lipid, suggesting that the lack of support for our hypotheses was due to real biological phenomena, and not to low statistical power. Thus, although we manipulated physiological systems central to egg production, we did not observe the expected effects on reproductive performance (increased egg mass through increased yolk mass), suggesting that the determination of this phenotype (egg mass) is tightly regulated.

**Effect of estradiol on yolk precursors and egg composition**

At the 1-egg stage, the yolk precursor levels of treated birds showed greater variation than those of shams but were within the range observed in a larger sample of unmanipulated birds \((N=37; \text{ Christians and Williams, 1999})\). Thus, exogenous estradiol elevated vitellogenin levels within the range of natural variation, i.e. its effect was physiological rather than pharmacological. The effect of exogenous estradiol appeared to persist throughout laying; the plasma levels of both vitellogenin and VLDL were elevated in estradiol-treated females at clutch completion compared to controls.

Despite the marked elevation of the circulating levels of yolk precursors, estradiol did not increase egg mass as predicted. This lack of effect was not due to low statistical power; the mass of the first egg of the replacement clutch tended to be lighter in estradiol-treated females, although this difference was not significant. Furthermore, estradiol did decrease the mass of yolk protein and lipid in eggs by 11% and 13%, respectively, suggesting that estradiol may have actually decreased the uptake of yolk precursors by the growing follicles. The accelerated regression of the ovaries at clutch completion in estradiol-treated females might also have been caused by depressed ovarian uptake of yolk precursors throughout laying. In addition, reduced precursor uptake provides an alternative explanation for the elevated plasma concentrations of the yolk precursors in estradiol-treated females. Higher precursor levels may not be due to increased precursor production by the liver, but instead may be the result of lower uptake rates at the ovary, and hence slower depletion of the circulating pools.

Follett and Redshaw (1974) reported that estradiol decreased the accumulation of radioactive vitellogenin by the ovaries of *Xenopus*, whereas estradiol together with follicle stimulating hormone (FSH) substantially increased precursor uptake. These authors speculated that estradiol may have lowered endogenous secretion of FSH by the pituitary (Follett and Redshaw, 1974), thereby reducing the endocrine (FSH) stimulation of oocyte growth. Negative feedback could be one mechanism by which egg mass is regulated within individual females, and may have buffered against exogenous estradiol, such that there were few effects on reproductive performance.

Variation in the rate of yolk precursor uptake provides a potential mechanism of egg mass determination. Investigation of this mechanism will require direct measurement and manipulation of uptake rates, e.g. by administration of FSH (cf. Follett and Redshaw, 1974). Palmer and Bahr (1992) found that exogenous FSH increased yolk deposition into the yolky follicles of chickens (*Gallus gallus domesticus*), particularly at low doses. In contrast, Sinervo and Licht (1990) found that FSH decreased egg mass in side-blotched lizards (*Uta stansburiana*), perhaps by increasing the number of follicles recruited into rapid yolk development and thereby increasing competition between follicles for circulating vitellogenin.

**Effect of estradiol on reproductive organs**

We hypothesized that exogenous estradiol would increase the hepatic production of the yolk precursors and so lead to hypertrophy of the liver. Treatment with estradiol did elevate plasma concentrations of vitellogenin and VLDL, either through increased production and/or decreased ovarian uptake (see above), but had no effect on the mass of the liver at the 1-egg stage or at clutch completion. This result is consistent with the finding that estradiol treatment of non-breeding zebra finches (*Taeniopygia guttata*) increased plasma precursor levels but did not lead to hypertrophy of the liver (Williams and Martyniuk, 1999). Although Rosebrough et al. (1982) found that estradiol treatment increased the mass of liver protein in turkey hens, this may have been a pharmacological effect of the high dosage used \((10 \mu g g^{-1})\).
As was the case with the liver, treatment with exogenous estradiol did not increase oviduct mass at the 1-egg stage, perhaps because circulating levels of endogenous hormone are naturally high at this time (Dawson, 1983). Although estradiol has been found to have dose-dependent effects on oviduct mass in non-breeders (e.g. Williams and Martyniuk, 1999), its effects on laying females, and the significance of natural inter-individual variation in estradiol levels in such birds, are not known. During breeding, negative feedback systems may operate to reduce dose-dependent effects, so that estradiol levels above some threshold stimulate complete growth of the oviduct. Such regulation of oviduct mass may explain why we did not observe an effect of estradiol on the mass of albumen protein. We did observe an effect of estradiol on oviduct mass at clutch completion, when endogenous estradiol levels would have begun to decline (Dawson, 1983). Similarly, other workers have found that estradiol treatment causes oviduct growth in immature or non-breeding females (Yu et al., 1971; Williams and Martyniuk, 1999), in which endogenous estradiol levels are relatively low.

**Effect of estradiol on endogenous nutrient stores**

Lean dry flight muscle mass was lighter in estradiol-treated females than in controls at the 1-egg stage. These findings could be interpreted in terms of increased protein mobilization to facilitate increased vitellogenin production by the liver. However, although treatment effects were observed during the peak of egg production effort, no differences were found at clutch completion, i.e. when the cumulative costs of egg production had been incurred. In a number of passerine species, flight muscle mass has been observed to decrease around the 1-egg stage in unmanipulated females (e.g. Jones and Ward, 1976; Houston et al., 1995), and so estradiol treatment may have hastened this process rather than increased the magnitude of muscle mass loss. Total body lipid did not differ between treatments at the 1-egg stage or clutch completion, again suggesting that exogenous estradiol did not lead to depletion of endogenous nutrient stores.

**Conclusions**

We predicted that exogenous estradiol would increase egg mass in European starlings. Although estradiol elevated the plasma concentrations of the yolk precursor vitellogenin, treatment did not increase egg mass or other measures of reproductive performance. The lack of support for our prediction was not due to a lack of statistical power; the mass of yolk protein and lipid was actually lighter in estradiol-treated birds. Lighter yolks suggest that estradiol may have decreased the uptake of yolk precursors at the ovary, perhaps via negative feedback effects on FSH secretion. The failure of exogenous estradiol to increase egg mass indicates that this phenotype may be regulated at higher levels of organization (e.g., negative feedback endocrine systems, uptake of yolk precursor at the ovary) than those studied in this experiment.

We thank the staff of the Pacific Agri-food Research Centre in Agassiz, British Columbia, for permission to work on their grounds and for providing a supportive atmosphere. J. J. Aiken provided invaluable support in the field and in the laboratory and D. Maney supplied helpful technical advice. E. M. Birmingham and two anonymous reviewers offered constructive comments on an earlier draft of the paper. This study was funded by an operating grant to T.D.W. from the Natural Sciences and Engineering Research Council of Canada (NSERC), and by NSERC postgraduate scholarships to J.K.C.

**References**


Effects of estradiol on egg production


