ELSEVIER

Available online at www.sciencedirect.com

SCIENCE DIRECT®

Hormones and Behavior 47 (2005) 585–591

Hormones and Behavior

www.elsevier.com/locate/yhbeh

Negative effects of elevated testosterone on female fecundity in zebra finches

Joanna Rutkowska[a,*], Mariusz Cichoń[a], Marisa Puerta[b], Diego Gil[c]

[a]*Institute of Environmental Sciences, Jagiellonian University, Gronostajowa 7, 30-387 Kraków, Poland*
[b]*Departamento de Fisiología (Fisiología Animal II), Facultad de Biología, Universidad Complutense de Madrid, 28040 Madrid, Spain*
[c]*Departamento de Ecología Evolutiva, Museo Nacional de Ciencias Naturales, José Gutiérrez Abascal, 2, 28006 Madrid, Spain*

Received 7 May 2004; revised 31 August 2004; accepted 9 December 2004
Available online 2 March 2005

Abstract

Although factors influencing androgen deposition in the avian egg and its effects on nestling fitness are recently receiving considerable attention, little is known about the potential costs of high testosterone levels in the females. Our study aimed at determining the effect of injections of testosterone (T) in female zebra finches (*Taeniopygia guttata*), on clutch size, egg mass, yolk mass, and yolk androgen content. Females were given a single bolus injection of T in a range of doses after laying the first egg. Results show that administration of T negatively affected clutch size; the strength of this effect increased with increasing doses of T. Females injected with the highest testosterone dose showed suppressed oviposition of the third and the fourth eggs. Interestingly, testosterone administration made females produce eggs with relatively large yolks, suggesting that T may mediate the trade-off between number and size of eggs. Testosterone injection resulted in elevated levels of androgen in the eggs, in contrast to control clutches, which showed a decreasing pattern of androgen concentration along the laying sequence. We conclude that high androgen investment in eggs may be limited by physiological requirements of the ovulatory process.

Keywords: Maternal investment; Trade-off; Egg size; Androgens; *Taeniopygia guttata*

Introduction

The role of androgens in female reproduction is a neglected research area (Staub and De Beer, 1997). Although several androgens have been identified in processes of follicle maturation and ovulation (Staub and De Beer, 1997), little is known about the functional significance of seasonal and individual variation in female androgen circulating levels. Recent interest in female androgen has been spurred by the discovery that females could influence offspring development and behavior by varying androgen deposition in avian yolks (Schwabl, 1993). Although factors influencing androgen deposition to the avian egg and their effects on nestling fitness are receiving increasing attention, little is known about the costs of such allocation in females and offspring (Gil, 2003).

The increased amount of yolk testosterone in eggs laid by females mated to attractive partners (Gil et al., 1999, 2004a) or in eggs of females encountering social stress (Groothuis and Schwabl, 2002; Reed and Vleck, 2001; Schwabl, 1997; Whittingham and Schwabl, 2002) suggests adaptive androgen allocation to eggs. These patterns make adaptive sense because of the positive effects of testosterone (thereafter: T) on offspring development, such as shortened embryo development (Eising et al., 2001), more vigorous begging behavior, faster growth rate, and higher social status once the bird achieves adulthood (Lipar and Ketterson, 2000; Schwabl, 1993, 1996a). However, such increased investment may also bring about some costs. Firstly, some offspring may not be able to bear high T levels. Indeed, in one study, high androgen levels inhibited nestling growth and survival (Sockman and Schwabl, 2000). The negative effects of elevated T level could also include increased oxidative stress (von Schantz et al., 1999), suppressed immune function (Da Silva, 1999; Folstad and Karter,

---

* Corresponding author.
E-mail address: rutko@eko.uj.edu.pl (J. Rutkowska).

0018-506X/$ - see front matter © 2005 Elsevier Inc. All rights reserved.
doi:10.1016/j.yhbeh.2004.12.006

1992), or elevated sibling aggression that may lead to non-adaptive brood reduction (Mock and Parker, 1997). Secondly, these costs may also be paid by the female if increased biosynthesis of egg androgens results in increased maternal levels of circulating androgens (Schwabl, 1996b) which may in turn have negative consequences for the female. Thus, females should optimize androgen deposition into the eggs taking into account their ability to cope with high androgen levels. Indeed, several studies reported the importance of female quality on yolk T allocation. For instance, androgen allocation increases with female age in the European starling (*Sturnus vulgaris*; Pilz et al., 2003) and decreases with increasing levels of developmental stress in the zebra finch (*Taeniopygia guttata*; Gil et al., 2004b).

Although several studies have shown a close relationship between female plasma and yolk T (Schwabl, 1996b; Whittingham and Schwabl, 2002), recent studies provide negative evidence, showing a much more confusing pattern. For instance, Mazuc et al. (2003) found a negative correlation between egg and plasma T in house sparrows, and Verboven et al. (2003) found that food supplementation of female gulls increased androgen levels in the plasma, whereas in another experiment food supplementation decreased yolk androgens.

One of the few studies that has experimentally manipulated T circulating levels in females showed that female red-winged blackbirds (*Agelaius phoeniceus*) with T implants presented impaired reproduction, with disruption of nest building and egg laying (Searcy, 1988). In the recent study on the spotless starling (*Sturnus unicolor*), females implanted with T showed 2-week delay in egg laying compared to control females (Veiga et al., 2004). The negative impact of exogenous androgens has also been reported in chickens (Brahmakshatriya et al., 1969). This evidence suggests that elevated androgens in females may have a negative effect on their fitness. While it is unlikely that those costs mentioned above occur naturally, subtler fecundity costs could be expected in females subjected to high T levels within the natural range. These fecundity costs could include reduction in clutch size or egg size. So far, correlative data do not provide support for these predictions, e.g., mean androgen level was positively related to clutch size in European starling (Pilz et al., 2003) and in barn swallows (*Hirundo rustica*, Gil et al., submitted for publication). However, life history theory predicts positive correlations among individuals in traits that are subject to trade-offs, since individual quality may mask expected negative correlations (van Noordwijk and de Jong, 1986). Experimental manipulations are necessary to uncover trade-offs among life history traits.

Our study aimed at determining the effect of a bolus injection of increasing amounts of T in females on clutch size, egg and yolk mass, and yolk-androgen content in birds. For our study, we chose zebra finches, a species that easily breeds in laboratory conditions, laying clutches that average 5–6 eggs. To ensure that T administration took place at the same precise stage of the reproductive cycle, we injected females on the day the first egg was laid. This allowed us to compare the effect of the treatment on androgen levels in subsequently laid eggs with respect to the first egg. If high levels of T are costly for the female, we expected that our treatment would negatively affect clutch size, egg mass, or yolk mass. Because at the time of injection the yolks of the third and forth eggs would be undergoing the most extensive growth (Christinas and Williams, 2001), we expected to detect the effects of T treatment mainly in the third and subsequent eggs.

Methods

Experimental design

Zebra finches originating from the laboratory colony were kept in a climatized room at 21 ± 2°C, under a 13:11 h light/dark photoperiod, lights on at 0700 h. Birds were fed ad libitum with a standard mixture of seeds (Megan, Poland), along with a mixture of hard-boiled egg chopped with finely grated carrot. Birds also received a cuttlebone and grit. Rearing conditions were kept constant during the experiment.

Initially, all birds were maintained in a common aviary, where they could mate freely and rear one brood. Sexes were then separated for 3 months and paired again in visually separated, individual cages (75 × 30 cm and 40 cm high) equipped with external nestboxes and nesting material.

Following pairing, nestboxes were inspected every morning between 0900 and 1000 h to record nest building and egg laying, as well as labeling new eggs. Freshly laid eggs were removed and replaced with clay models. The removed eggs were weighed (±0.01 g) and the yolk was separated from the albumen, weighed (±0.01 g), and frozen at −20°C. As we were not always able to separate entire yolk from the albumen, 16 yolks were not weighed. Laying gaps seldom occurred. Eggs laid after a gap (up to 3 days) were numbered as though the missing egg had been laid.

After laying the first egg, females received a subcutaneous T injection (*testosteronum enanthanum*, Jelfa S.A., Poland) in the inguinal region between 1000 and 1300 h. Females were randomly assigned to seven experimental groups. Sample sizes for each group are presented in Fig. 1. T-treated females received 2.5, 5, 10, 20, or 40 μg T dissolved in 50 μl of oil *(paraffinum liquidum*), respectively. Control females received vehicle only. Sham controls were only caught and removed from their cages for a few minutes. Females assigned to different experimental groups did not differ in body mass ($F(6,35) = 0.55$, $P = 0.77$) or in the mass of the first egg in a clutch ($F(6,35) = 0.92$, $P = 0.49$).