JOURNAL OF
AVIAN BIOLOGY

Article

Food restriction delays breeding and affects insulin-like growth factor-1, oxidative damage and haematocrit value before egg-laying in female canaries

Rita Hargitai, Nóra Boross, Zsófia Tóth and Ádám Z. Lendvai

R. Hargitai (https://orcid.org/0000-0002-5803-2393) ✉ (rita.hargitai@gmail.com) and N. Boross, Dept of Systematic Zoology and Ecology, Eötvös Loránd Univ., Budapest, Hungary. NB also at: Centre for Ecological Research, Balaton Limnological Inst., Tihany, Hungary. – Z. Tóth and Á. Z. Lendvai (https://orcid.org/0000-0002-8953-920X), Dept of Evolutionary Zoology and Human Biology, Univ. of Debrecen, Debrecen, Hungary. ZT also at: Juhász-Nagy Pál Doctoral School of Biology and Environmental Sciences, Debrecen, Hungary.

Journal of Avian Biology
2022: e02866
doi: 10.1111/jav.02866
Subject Editor: Suvi Ruuskanen
Editor-in-Chief: Staffan Bensch
Accepted 5 January 2022

Environmental challenges, such as fluctuations in food availability, could influence reproductive investment. If resource availability is poor, individuals need to decide in which life-history process they invest more energy and vital compounds, which determine the cost of reproduction. In resource allocation, the physiological pathways have important roles. The aim of our study was to examine whether food availability influenced physiological traits (insulin-like growth factor-1 (IGF-1) concentration, antioxidant capacity (OXY), level of oxidative damage (ROM) and haematocrit value) prior to egg-laying in female canaries *Serinus canaria*. We also tested whether these physiological traits were associated with traits reflecting the reproductive investment in egg macro- (egg mass and yolk mass) and micronutrient content (eggshell biliverdin- and protoporphyrin-based colouration). To test these questions we conducted a food restriction experiment with control and food-restricted (72% of control food) groups. Our study showed that food-restricted canary females delayed egg-laying and the physiological traits differed between the groups. At the pre-laying period, after 9–11 days of treatment, a reduction in plasma IGF-1 concentration, ROM level and haematocrit value was detected in the control group, whereas in the food-restricted group plasma IGF-1 concentration increased, while ROM level and haematocrit value did not change. Plasma level of OXY was not influenced by treatment or breeding period. Plasma concentration of IGF-1, haematocrit value and oxidative status before egg laying did not affect the egg characteristics. Our study highlights the importance of breeding stage when studying and interpreting the effects of food restriction on physiological traits of breeding birds. Moreover, our data suggest that nutritional limitation had an effect on the timing of egg-laying that could be mediated by changes in physiological variables.

Keywords: egg colouration, nutritional stress, oxidative shielding hypothesis, oxidative stress, reproductive investment, timing of egg laying

NORDIC SOCIETY OIKOS
www.avianbiology.org

Introduction

Environmental challenges, such as fluctuations in food availability before and during the breeding period, could influence reproductive investment. If resource availability is poor, individuals need to decide in which life-history process they invest more energy and vital compounds, which underlie the concept of the cost of reproduction (Stearns 1989). These resource allocation trade-offs are mediated by physiological traits, such as circulating levels of hormones and other important biomolecules, which are affected by several environmental factors, including food availability. These environmental factors could represent a proximate constraint and may exert an influence on the female's investment in the current reproduction, which in turn may affect the physiological status of females after breeding due to the cost of reproduction. Nonetheless, our knowledge is still scarce regarding the physiological mechanisms and molecular signalling pathways underlying such costs and constraints (Zera and Harshman 2001, Ricklefs and Wikelski 2002).

Food restriction may affect several physiological mechanisms, including the circulating levels of various hormones, proteins, lipids and micronutrients (Harvey et al. 1981, Totzke et al. 1999, Buyse et al. 2000), which could influence reproductive investment. An important circulating compound that is responsive to nutritional limitation and could exert an influence on reproduction is insulin-like growth factor-1 (IGF-1). IGF-1 is a pleiotropic peptide hormone regulating growth, body size, sexual maturation, reproductive investment and lifespan across a broad range of species (Baker et al. 1993, Holzenberger et al. 2003, Van Heemst et al. 2010, Dantzer and Swanson 2012, Lodjak et al. 2018, Lendvai et al. 2021, Lodjak and Verhulst 2020). Fasting or low dietary protein intake was shown to decrease plasma IGF-1 concentrations in various species including dairy cattle *Bos taurus* (Zulu et al. 2002), Steller sea lions *Eumetopias jubatus* (du Dot et al. 2009), domestic fowl *Gallus gallus domesticus* (Lauterio and Scanes 1987) and great tits *Parus major* (Lodjak et al. 2014). It was observed that passerine nestlings showed lower IGF-1 levels when food availability was limited, and this resulted in reduced growth rates and poorer body conditions (Lodjak et al. 2014, 2017). In contrast, under some circumstances, food restriction may induce an increase in IGF-1 levels, as reported in fish, chickens and humans (Hocking et al. 1994, Rasmussen et al. 1994, Ayson et al. 2007). The physiological effects of IGF-1 have also been suggested to vary according to body condition (Lodjak et al. 2016). In line with a context-dependent IGF-1 regulation, a recent study has reported that captive bearded reedlings *Panurus biarmicus* showed a highly plastic IGF-1 response to food restriction: IGF-1 levels either decreased, increased or did not change and this response remained consistent within individuals (Tóth et al. unpubl.).

IGF-1 plays an important role in the growth and maturation of reproductive tissues and gonadal steroidogenesis in fish (Weber and Sullivan 2000) and mammals (Daftary and Gore 2005, Pitetti et al. 2013). Moreover, it is associated with life-history variation in reptiles (Sparkman et al. 2009, 2010), birds (Lodjak et al. 2018) and mammals (Swanson and Dantzer 2014). However, very little is known about how IGF-1 levels influence reproductive traits in birds, and the few studies so far have focused on poultry (Hocking et al. 1994, McMurtry et al. 1997, Onagbesan et al. 1999, Kim et al. 2004). In free-living passerines, there was one study that detected an association between plasma IGF-1 levels and egg weight, and the direction of the association depended on the species' body weight (Lodjak et al. 2018). Furthermore, it was reported that IGF-1 could play a role in the development of sexually selected ornaments in a sexually dichromatic passerine species, the bearded reedling (Mahr et al. 2020).

Limited food intake is expected to reduce the availability of dietary antioxidants, which could result in lower antioxidant capacity and increased levels of oxidative stress (Catoni et al. 2008, Fletcher et al. 2013, Giordano et al. 2015), as well as a lower investment in reproduction (Costantini et al. 2016, Montoya et al. 2016). Reactive oxygen species (ROS), byproducts of normal aerobic metabolism, have deleterious effects on cellular biomolecules, and when they overcome the organism's ability to metabolize them by antioxidants, oxidative stress occurs (Finkel and Holbrook 2000, Speakman and Selman 2011). The antioxidant defence system acts through several pathways against the produced ROS, including endogenous, synthesized antioxidant molecules (e.g. enzymes, proteins) and exogenous, ingested antioxidants (e.g. carotenoids, vitamins, polyphenols; Surai 2002, Halliwell and Gutteridge 2007). Limited protein availability could lead to elevated levels of oxidative damage and decreased antioxidant capacity, probably through reduced levels of glutathione and uric acid (Cohen et al. 2009, Swennen et al. 2011, Alan and McWilliams 2013), as well as through the decline in the synthesis of some antioxidant enzymes (Feoli et al. 2006). Therefore, food-restricted individuals may experience a lower total antioxidant capacity. Nonetheless, caloric restriction could decrease mitochondrial ROS production, reducing the level of oxidative damage (Sohal et al. 1994, Yu 1996, Barja 2002). Therefore, although the antioxidant capacity may be reduced due to nutrient limitation in food-restricted animals, it is unclear whether the level of oxidative stress is elevated.

The environmental influence on haematocrit value, a measure of the relative volume of red blood cells compared with total blood volume, was also demonstrated in experiments where nutritional stress decreased the haematocrit value (Boismenu et al. 1992, Merilä and Svensson 1995, Webb et al. 2017). Haematocrit value also varies in female birds during the breeding period. Erythropoiesis is generally suppressed, while blood plasma volume is increased during egg laying due to the effects of oestrogen hormones, causing reproductive anaemia of egg-producing females (Morton 1994, Williams et al. 2004, Wagner et al. 2008).

The aim of our study was to examine in female canaries *Serinus canaria* whether experimental food restriction prior to egg laying influenced physiological traits that could be related to the nutritional condition of the female and that may also affect her reproductive decisions. For this purpose

we conducted new analyses based on a dataset obtained during our earlier food-restriction experiment (Hargitai et al. 2018). In that study, we found that food limitation did not affect yolk mass and eggshell blue-green colouration, but it increased the intensity of brown eggshell colouration in canaries (Hargitai et al. 2018); therefore, the effects of treatment on egg traits are not presented in this paper. Here, we studied four physiological variables that may be affected by nutritional condition: circulating IGF-1 level, plasma antioxidant capacity, plasma level of oxidative damage and haematocrit value; these variables were not examined in our earlier paper of the same experiment (Hargitai et al. 2018). We tested whether these physiological traits were associated with traits reflecting the reproductive investment in egg macro- (egg mass and yolk mass) and micronutrient content (eggshell biliverdin- and protoporphyrin-based colouration). Biliverdin pigment causes the blue-green colouration of bird eggs, while red-brown maculation is caused by protoporphyrin pigment (Kennedy and Vevers 1976, Mikšík et al. 1996). Biliverdin shows an antioxidant activity (McDonagh 2001, Kaur et al. 2003), whereas protoporphyrin possesses pro-oxidant properties (Afonso et al. 1999).

We hypothesised that food-restricted females would have reduced plasma IGF-1 level, antioxidant capacity and haematocrit value than control females, but it is unclear how the treatment would change the level of oxidative damage. Moreover, we expected that plasma IGF-1 level and antioxidant capacity at the pre-laying period would be positively related to reproductive investment of female birds. Haematocrit value before reproduction may be suppressed (Morton 1994, Williams et al. 2004, Wagner et al. 2008), but it may also show a positive association with nutritional condition (Boismenu et al. 1992, Merilä and Svensson 1995, Webb et al. 2017), therefore, the direction of its association with reproductive investment is not clearly predictable. To our knowledge, the associations among plasma physiological variables during the pre-breeding period and their possible relationships with reproductive investment under food restriction have not yet been investigated in a songbird species.

Material and methods

Animals and housing conditions

The experiment was conducted with 2–4 year-old domesticated canaries at Eötvös Loránd University, Budapest, Hungary, in 2015 (details in Hargitai et al. 2018). Birds were kept at 23°C and under natural light conditions supplemented with artificial light in a light cycle of 12L:12D. Water, cuttlefish bone and grit were provided ad libitum to the birds. All birds were identified with a numbered ring. Females were kept in individual cages (45 × 27 × 43 cm), while males were kept individually out of the breeding period (cage size: 40 × 25 × 32 cm), but shared a cage with a female during breeding. The National Food Chain Safety Office (NÉBIH) provided permissions for this study (PEI/001/824-4/2015). All applicable international, national and institutional guidelines for the care and use of animals were followed.

Experimental design

We weighed females with a Pesola spring balance (to the nearest 0.1 g), and measured the length of their tarsus with a calliper (to the nearest 0.1 mm) before the start of the experiment. We assigned females to one of the two groups (20 treated and 15 control) so that the average body mass, tarsus length and distribution of age categories were similar in the two groups. We included more females in the treated group as we expected lower egg-laying activity due to food restriction (Meijer and Langer 1995). All birds were maintained on a standard diet for canaries (a mixture of grains; DaCapo Züchter-spezial, Hesa). Control females received 4.5 g of seed and 1 g of grated boiled egg white per day, while food-restricted females received 3.25 g of seed (72% of control food) and 0.25 g of grated boiled egg white per day (Hargitai et al. 2018). Prior to the experiment ('before treatment') we weighed all females and we took blood samples (50–200 μl) by puncturing the brachial vein. We collected blood samples in heparinized capillaries, and centrifuged the capillaries at 10 000 g for 10 min. We separated plasma from cells and stored the plasma at −80°C until later analyses. We weighed females again and took a second blood sample 9–11 days after the beginning of the treatment ('pre-laying period') (females were not sampled on the same day due to logistical reasons). We found that body condition showed a more pronounced reduction in the treated group than in the control group after the beginning of the experiment (Hargitai et al. 2018).

Then, we provided a nest pan with felt liner and nesting material, and placed a male canary in the cage. Each male bird was paired with one control and one treated female, and males were switched every two days between the female pairs. If no egg-laying occurred in the treated female during two weeks, we introduced the male into the cage of another treated female. Males were removed from the cage when egg laying was finished.

We checked the cages daily to determine the initiation of egg laying and the laying order of the eggs. A higher proportion of control females started egg-laying than food-restricted females (Hargitai et al. 2018). We numbered the eggs with a waterproof marker according to laying order and they were collected on the day of laying. Collected eggs were replaced by plastic dummy eggs of similar size and appearance, which are regularly used by breeders as well. We collected whole clutches (1–4 eggs; mean: 2.0 eggs); the clutch size distribution was as follows: 1 egg: n = 6 nests, 2 eggs: n = 7 nests, 3 eggs: n = 5 nests, 4 eggs: n = 1 nest. We weighed the eggs with a balance (to the nearest 0.01 g) and measured eggshell colouration with a spectrophotometer. We opened the eggs and weighed the yolk (to the nearest 0.01 g).

Eggshell spectrophotometric analyses

Canaries lay blue-green eggs speckled with brown pigment spots. Eggshell colouration was measured using a portable spectrometer with a bifurcated fibre-optic probe (QR400-7-SR-BX, Ocean Optics Europe) and a DH-2000 deuterium-halogen light source (Ocean Optics Europe) on three background and three spotted areas, as described in a previous study (Hargitai et al. 2018). OOIBase32 software (Ocean Optics Europe) was used to record the reflectance spectra. We calculated blue-green chroma as $(R_{480nm}-R_{370nm})/R_{480}$ because the eggshell spectra showed peak reflectance at 480 nm and lowest reflectance at 370 nm (Hargitai et al. 2016), which corresponds to the absorption spectra of biliverdin (Singleton and Laster 1965). Brown chroma was calculated as $R_{600–700nm}/R_{320–700nm}$, as protoporphyrin reflects the light at this spectrum region (König and Meyer 1992), while biliverdin has a reflectance minimum around 660 nm (Singleton and Laster 1965). In previous studies, it was shown that eggshell biliverdin concentration positively correlated with eggshell background blue-green chroma (Hargitai et al. 2018), while eggshell protoporphyrin concentration correlated with eggshell spot brown chroma (Hargitai et al. 2016).

Plasma IGF-1 concentration analyses

Plasma IGF-1 levels were measured by a competitive ELISA at the University of Debrecen described in detail in Mahr et al. (2020). Briefly, 96-well microplates were coated at 4°C overnight with 100 µl of an antibody raised against IGF-1 in rabbits. The coated plate was incubated for 2 h at room temperature with either 20 µl of standard (known concentrations of synthetic chicken IGF-1 in serial dilutions starting at 500 ng ml^{-1}) or 20 µl of sample and 100 µl of biotinylated IGF-1 as a tracer. After incubation, the microplate was washed three times with 250 µl of PBS containing 0.025% Tween 20. After washing, 100 µl of streptavidin-horseradish peroxidase conjugate was added to all wells and incubated at room temperature for 30 min. After washing, 100 µl of tetra-methyl-benzidine was added to the wells and incubated at room temperature for 30 min. The enzymatic reaction was stopped by adding 100 µl of 1 M H_2SO_4, and optical density was measured at 450 nm (reference at 620 nm) using a microplate reader.

Plasma antioxidant capacity test

Total plasma antioxidant capacity was measured by the OXY-Adsorbent test (Diacron, Grosseto, Italy). The OXY test measures the ability of the antioxidant compounds of the plasma (e.g. carotenoids, vitamins A, C and E, proteins, thiols) to cope with the oxidizing action of hypochlorous acid (HOCl). Samples were tested in duplicates and results were expressed as millimolar HOCl neutralized (see for details Hargitai et al. 2016). Mean intraplate coefficient of variation was 2.4%, mean inter-plate coefficient of variation was 9.0%.

Plasma oxidative damage test

Plasma levels of reactive oxygen metabolites (ROMs, primarily hydroperoxides, ROOH) were measured by the d-ROM assay (Diacron International, Grosseto, Italy), which is a reliable assay for the quantification of plasma oxidative damage (Costantini 2016). ROMs are intermediate oxidative damage compounds, which are generated by the peroxidation of macromolecules by ROS. We did not use duplicates of samples, as the CV of the serum standards was very low (mean: 1.6%), and previous studies also showed similarly low CV for bird plasma samples (Hargitai et al. 2016), and we had limited amount of plasma. ROM concentration was calculated using a calibration curve of lyophilized serum standard supplied by the manufacturer. Plasma ROM concentration is expressed as mM of hydrogen peroxide (H_2O_2) equivalents (details in Hargitai et al. 2016).

Haematocrit value

Capillary length occupied by red blood cells or total blood was measured following centrifugation (10 000 g for 10 min) with a ruler (to the nearest 0.5 mm), and haematocrit value was calculated as capillary length occupied by red blood cells divided by capillary length occupied by total blood. If blood was collected to more than one capillary from a bird at a given sampling event, average haematocrit value was calculated.

Statistical methods

Sample sizes differ for each physiological trait as we could not measure all of them from each blood sample. Number of days (9, 10 or 11) that elapsed from the beginning of the treatment until the second sampling event (pre-laying period) had no significant effect on the measured physiological variables at that period (IGF-1: $p=0.71$; OXY: $p=0.07$; ROM: $p=0.12$; haematocrit: $p=0.27$), therefore, we did not include this variable in further models. We analysed the effect of treatment on the time to egg laying (the number of days that elapsed from the beginning of the treatment until the laying of the first egg) with a general linear model. Altogether 11 control and 8 treated females started egg laying, these birds were regarded as 'breeders'.

We tested the effects of treatment and breeding period (before treatment versus at 9–11 days of treatment or pre-laying period) as fixed factors on plasma levels of IGF-1, antioxidant capacity (OXY), oxidative damage (ROM) and haematocrit value with general linear mixed models (GLMMs), including female breeding status (breeder versus nonbreeder) as a cofactor, and the interaction between the fixed factors, entering female identity as a random factor. The effects of breeding status were non-significant in all cases (not shown).

We tested the relationships between female physiological traits at the pre-laying period and the number of days that elapsed from the pre-laying period until egg-laying with general linear models (GLMs) with female trait (plasma IGF-1 concentration, plasma antioxidant capacity, plasma level of oxidative damage, haematocrit value) as dependent variable,

number of days and treatment as fixed effects, including the interaction between fixed effects.

The relationships between haematocrit value and female physiological traits (IGF-1, OXY, ROM) were tested with GLMMs, including treatment, breeding period (pre-treatment versus pre-laying periods), breeding status, and a female physiological trait as covariate, and interactions between predictor variables in the models. Female identity was the random factor. Associations between IGF-1 level and plasma levels of OXY and ROM were also tested in similar models.

We analysed the relationships between female physiological traits (plasma IGF-1 concentration, plasma antioxidant capacity, plasma level of oxidative damage, haematocrit value) and reproductive investment with GLMMs with the clutch mean of an egg trait (egg mass, yolk mass, eggshell background blue-green chroma, eggshell spot brown chroma) as the dependent variable, treatment as factor and a female physiological trait at the pre-laying period as a continuous predictor variable, including the interaction between predictor variables. Number of days that elapsed from the pre-laying period until egg laying and clutch size were also included as covariates in the models. We performed separate models of each egg trait with each female trait, as sample size was not high enough to include all female traits in one model. Female identity was the random factor. Two-way interactions between predictor variables were included in the models, but all of them were not significant (not shown). We also tested the association between female physiological traits and clutch size using GLMs with the same predictor variables as in the GLMMs above.

In all models, backward stepwise selection procedure was used, removing non-significant variables from the model one by one in decreasing order of p-value. Normality of the residuals was checked with Shapiro–Wilk test and graphical verification, homoscedasticity was tested with Levene's test. Non-significant variables were re-introduced to the final model one by one, and these F- and p-values were presented. Analyses were performed in SPSS ver. 19.0 and STATISTICA ver. 5.5.

Results

Effect of food restriction on the timing of breeding

We found that treatment delayed egg laying ($F_{1,16}=4.55$, $p=0.049$; control group: 20.9 ± 6.6 days; food-restricted group: 27.6 ± 6.7 days).

Effects of food restriction and breeding period on female physiological traits

IGF-1

We found a significant interaction between treatment and breeding period on plasma IGF-1 concentrations ($F_{1,26.13}=16.95$, $p < 0.001$). Before treatment, there was no difference in plasma IGF-1 concentrations between the groups ($F_{1,25}=1.29$, $p=0.27$; control: $n=11$, treated: $n=14$). At the pre-laying period, we found a significant difference between the control and treated groups ($F_{1,26}=6.27$, $p=0.019$; control: $n=12$, treated: $n=14$): IGF-1 levels were significantly lower in the control group than in the food-restricted group (Fig. 1). We found that plasma IGF-1 levels declined between the pre-treatment and pre-laying periods in the control group ($F_{1,11.37}=8.66$, $p=0.013$), but showed a significant increase in the food-restricted group ($F_{1,15.02}=8.95$, $p=0.009$; Fig. 1).

We also observed that female plasma IGF-1 level at the pre-laying period showed a negative association with the number of days until egg-laying, indicating that females with lower IGF-1 levels laid eggs sooner ($F_{1,14}=9.64$, $p=0.008$; beta = −0.64, $n=16$; Fig. 2). Plasma IGF-1 level and haematocrit value did not correlate ($F_{1,41.9}=1.07$, $p=0.31$). In addition, we tested the correlation of plasma IGF-1 level with plasma antioxidant capacity and oxidative damage level, but neither plasma antioxidant capacity (OXY: $F_{1,48}=0.01$, $p=0.91$), nor plasma level of oxidative damage (ROM: $F_{1,33.9}=0.09$, $p=0.76$) showed a significant association with plasma IGF-1 concentration.

OXY

We found no significant interaction between treatment and breeding period on plasma level of antioxidant capacity (OXY; $F_{1,58}=0.70$, $p=0.41$; control, before treatment: $n=13$; control, pre-laying period: $n=12$; treated, before treatment: $n=16$; treated, pre-laying period: $n=17$). There was no difference in plasma antioxidant capacity between treatment groups ($F_{1,58}=0.53$, $p=0.47$) and breeding periods ($F_{1,58}=0.77$, $p=0.38$). We observed no significant relationship between plasma level of antioxidant capacity and the number of days until egg-laying from the pre-laying period ($F_{1,17}=1.25$, $p=0.28$, $n=19$). Haematocrit value and OXY level showed a marginally significant negative association ($F_{1,31.9}=3.90$, $p=0.057$).

ROM

We found a significant interaction between treatment and breeding period on plasma level of oxidative damage ($F_{1,22.38}=4.82$, $p=0.039$; Fig. 3). Before treatment, there was no difference in the plasma level of oxidative damage between the groups ($F_{1,25}=0.01$, $p=0.96$; control: $n=11$, treated: $n=14$). At the pre-laying period, the plasma level of oxidative damage was significantly lower in the control group than in the food-restricted group ($F_{1,18}=7.03$, $p=0.016$; control: $n=12$, treated: $n=7$). We found that plasma level of oxidative damage decreased significantly between the two periods in the control group ($F_{1,9.91}=19.27$, $p=0.001$), but showed no significant change in the food-restricted group ($F_{1,13.41}=0.01$, $p=0.92$; Fig. 3).

We detected no significant relationship between plasma level of oxidative damage and the number of days until egg-laying at the pre-laying period ($F_{1,13}=2.66$, $p=0.13$, $n=15$). Plasma level of oxidative damage and haematocrit value showed a marginally significant correlation before treatment ($F_{1,22}=4.15$, $p=0.054$, beta = 0.40), and a strong positive correlation at the pre-laying period ($F_{1,16}=45.23$, $p < 0.001$, beta = 0.80; interaction between ROM level and breeding period: $F_{1,12.5}=9.63$, $p=0.009$).

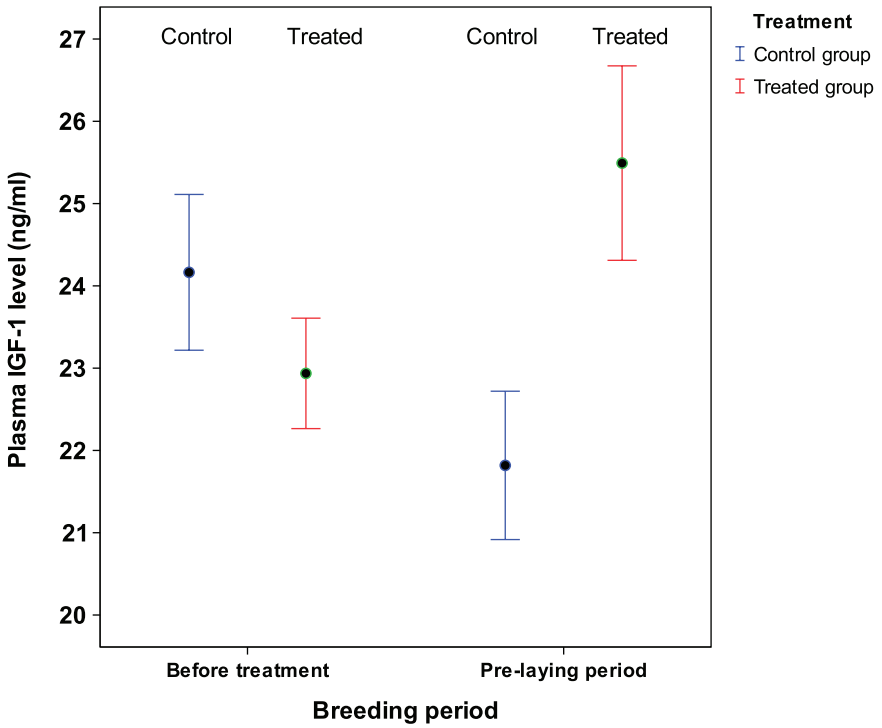

Figure 1. Mean ± SE of plasma insulin-like growth factor-1 (IGF-1) concentration (ng ml^{-1}) in female canaries of control (blue) and treated (food-restricted; red) groups at the first sampling event (before treatment) and the second sampling event (pre-laying period, after 9–11 days of treatment).

Haematocrit value

We found a significant interaction between treatment and breeding period on female haematocrit value ($F_{1,33}=6.39$, $p=0.016$). Before treatment, there was no difference in the haematocrit value between the groups ($F_{1,33}=0.50$, $p=0.49$; control: $n=13$, treated: $n=20$). At the pre-laying period, the haematocrit value was significantly lower in the control group than in the food-restricted group ($F_{1,33}=4.57$, $p=0.040$; control: $n=13$, treated: $n=20$; Fig. 4). Haematocrit value declined significantly between the two periods in the control group ($F_{1,13}=24.99$, $p < 0.001$), but showed no significant change in the food-restricted group ($F_{1,20}=2.91$, $p=0.10$).

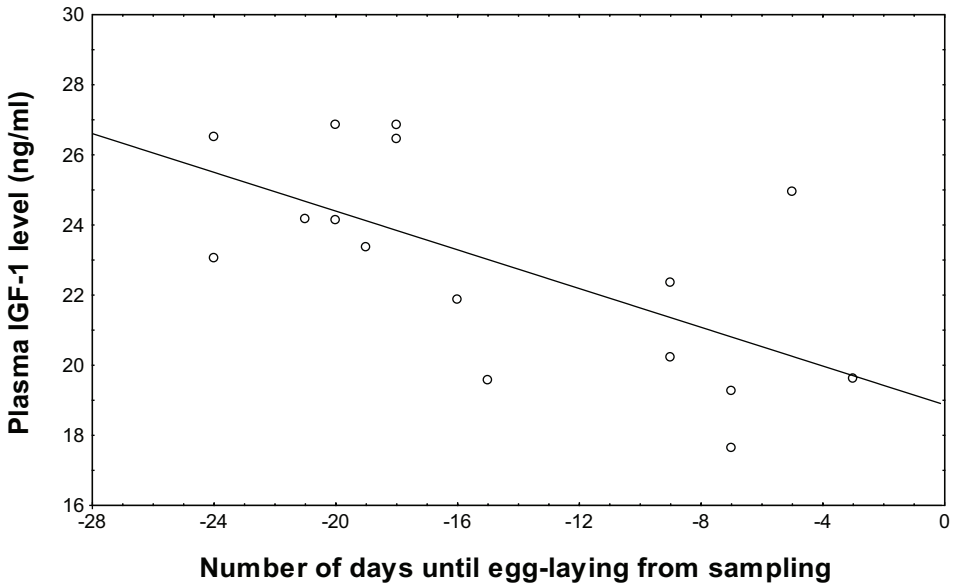

Figure 2. Relationship between number of days until egg laying (laying date = 0) from the second sampling event (pre-laying period) and plasma insulin-like growth factor-1 (IGF-1) concentration (ng ml^{-1}) at the pre-laying period in female canaries.

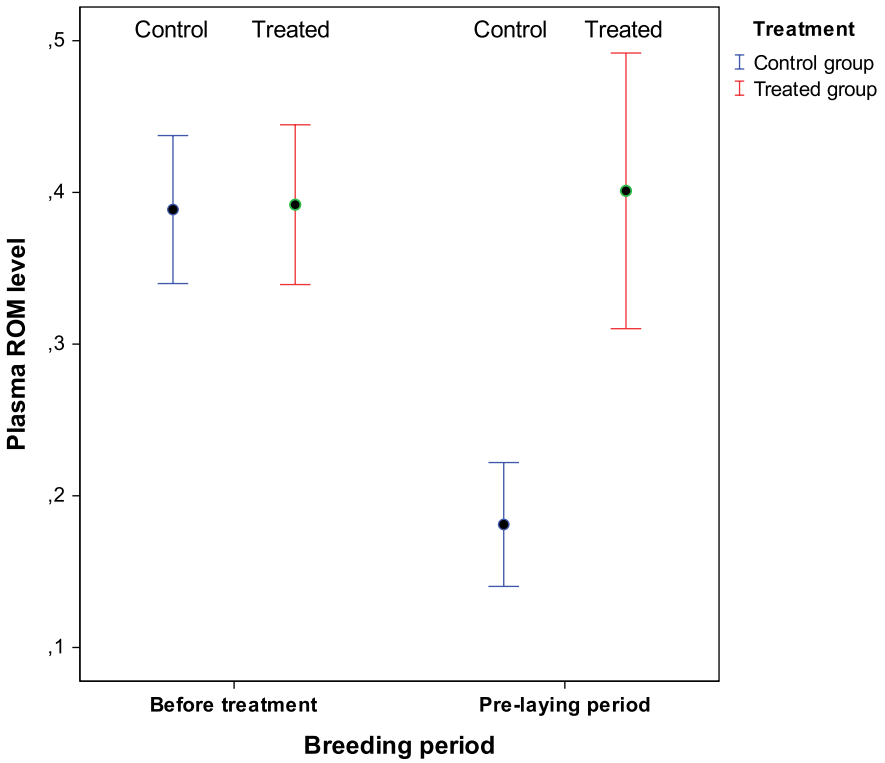

Figure 3. Mean ± SE of plasma level of oxidative damage (ROM; mM of H_2O_2) in female canaries of control (blue) and treated (food-restricted; red) groups at the first sampling event (before treatment) and the second sampling event (pre-laying period, after 9–11 days of treatment).

We also detected that haematocrit value at the pre-laying period showed a negative association with the number of days until egg-laying in the control group, indicating that control females with lower haematocrit value laid eggs sooner ($F_{1,9}=22.81$, $p=0.001$; beta$=-0.85$, $n=11$; Fig. 5), but this relationship was not significant in the food-restricted group ($F_{1,6}=1.23$, $p=0.31$, $n=8$; Fig. 5; interaction between treatment and number of days until egg-laying: $F_{1,15}=8.48$, $p=0.011$).

Relationships between female physiological traits and reproductive investment

We found no significant associations between clutch size, the clutch mean of an egg trait (egg mass, yolk mass, eggshell background blue-green chroma, eggshell spot brown chroma) and female physiological traits, such as plasma IGF-1 concentration (all $p > 0.71$), plasma antioxidant capacity (OXY; all $p > 0.17$), plasma level of oxidative damage (ROM; all $p > 0.11$) and haematocrit value (all $p > 0.11$) (see full models in the Supporting information). In these models, the effects of clutch size and the number of days between pre-laying sampling and laying date were not significant (Supporting information). Treatment had a significant effect only on eggshell spot brown chroma (Supporting information), as already reported in Hargitai et al. (2018).

Discussion

Our study showed that in response to food restriction canary females delayed egg-laying, similarly as it was reported in food-restricted poultry hens (Hocking et al. 1994, Eitan et al. 1998). Moreover, we found differences in physiological traits between treated and control females at the pre-laying period. Reductions in plasma IGF-1 concentrations, ROM levels and haematocrit values were detected in the control group before egg-laying, but in the food-restricted group, plasma IGF-1 concentrations increased, while ROM levels and haematocrit values did not change. We suggest that the detected differences in plasma IGF-1 concentrations, ROM levels and haematocrit values between the control and food-restricted groups were related to differences in their breeding stages that were influenced by food availability.

Control canary females at the pre-laying period had lower plasma IGF-1 concentration than 9–11 days earlier and we also found that females with lower IGF-1 levels at the pre-laying period laid eggs sooner. These results suggest that circulating IGF-1 level declines as laying date approaches, which seems to be in contrast to the findings that IGF-1 plays an important role in the growth and maturation of reproductive tissues and gonadal steroidogenesis in several taxa (Weber and Sullivan 2000, Daftary and Gore 2005, Pitetti et al. 2013). We presume that circulating oestradiol levels increased in canary females as laying date

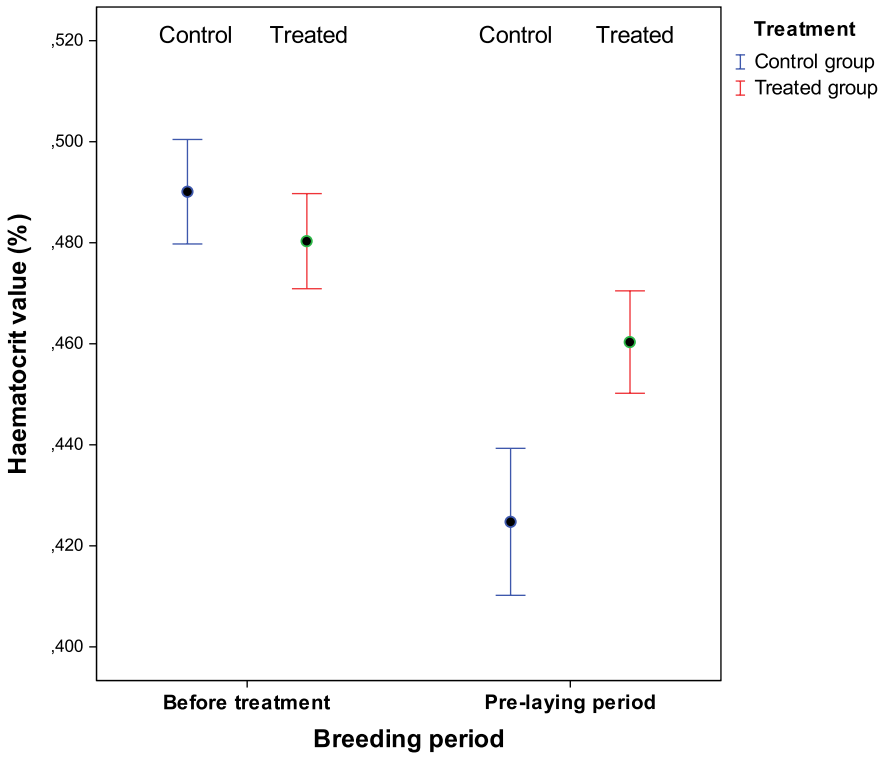

Figure 4. Mean ± SE of haematocrit value (%) in female canaries of control (blue) and treated (food-restricted; red) groups at the first sampling event (before treatment) and the second sampling event (pre-laying period, after 9–11 days of treatment).

approached similarly as shown in starlings *Sturnus vulgaris* at the period of nest-building (Dawson 1983) and domestic hens before egg laying (Eitan et al. 1998), although we did not measure plasma oestradiol levels in this study. A direct inhibitory effect of oestrogen on hepatic IGF-1 production, and consequently on plasma IGF-1 concentration was previously demonstrated in humans (Helle et al. 1996, Ho et al. 1996, Malarkey et al. 1997), and it was also found that IGF-1 level declined prior to egg-laying in snakes (Sparkman et al. 2010).

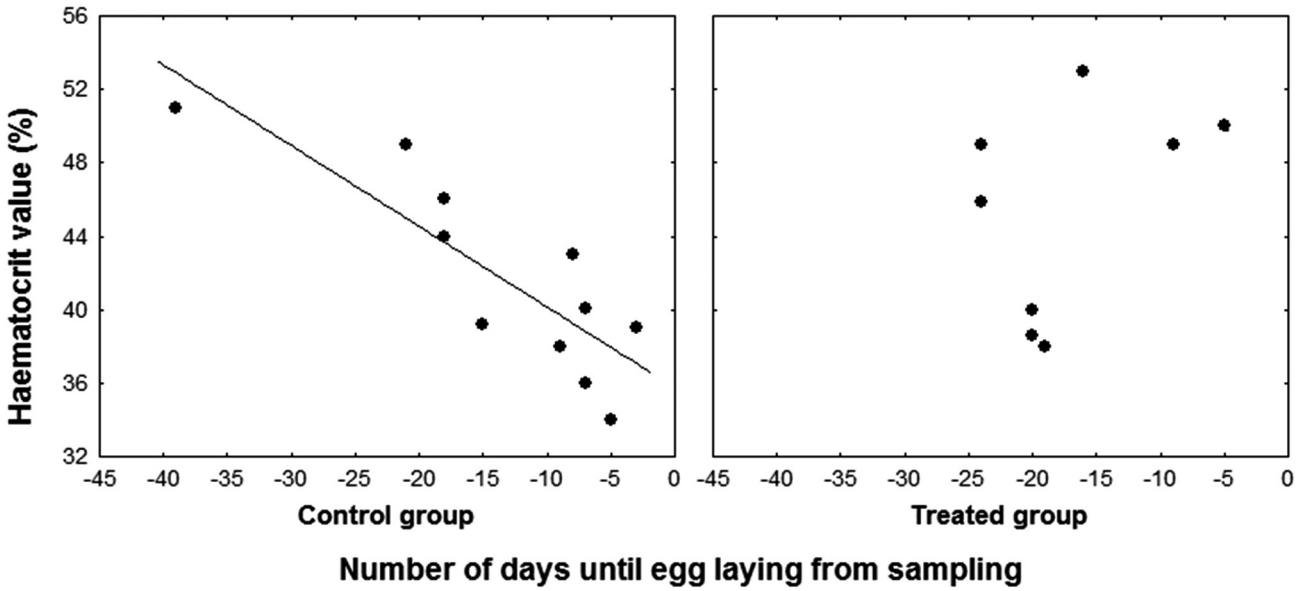

Figure 5. Relationship between number of days until egg laying (laying date = 0) from the second sampling event (pre-laying period) and haematocrit value (%) at the pre-laying period in female canaries. The relationship was significant in the control group ($p = 0.001$), but not in the food-restricted group ($p = 0.31$).

Food restriction delayed egg laying in canaries and also resulted in higher plasma IGF-1 concentration at the pre-laying period. Similar to our findings, food restriction resulted in high plasma IGF-1 concentration and delayed egg laying as compared to ad libitum fed control birds in broiler chickens (Hocking et al. 1994). Moreover, the expression of IGF-1 in the liver of food-restricted fish was significantly higher 3–6 days after starvation than in control fish (Ayson et al. 2007). It needs further studies to elucidate the physiological mechanisms that caused these differences due to food restriction. A possible mechanism may be that food restriction delayed the increase in oestrogen production, similarly as it was reported in poultry (Eitan et al. 1998, Onagbesan et al. 2006), and thus, the inhibitory effect of oestrogen on IGF-1 production is reduced, leading to higher circulating IGF-1 concentrations. This hypothesis requires further investigations.

In contrast to our expectations, food restriction did not alter plasma antioxidant capacity (OXY) of female canaries. By contrast, other studies reported that reduced food intake resulted in lower antioxidant capacity in birds and mammals (Feoli et al. 2006, Cohen et al. 2009, Swennen et al. 2011, Alan and McWilliams 2013, Fletcher et al. 2013, Giordano et al. 2015). It is possible that in the case of lower dietary antioxidant uptake, female canaries utilized their antioxidant stores and allocated them to the circulation (Monaghan et al. 2009).

We observed that plasma level of oxidative damage (ROM) of female canaries declined in the control group between the pre-treatment and pre-laying periods. In a previous study of canaries (Hargitai et al. 2016), a reduction in ROM level between the pre-laying and egg-laying periods was also observed, while plasma level of OXY remained constant. Our results showed that the decline in ROM value in the pre-laying period in the control group was related to the reduction in haematocrit value. As haematocrit value declines before breeding due to a higher blood plasma volume (Williams et al. 2004), the decline in plasma level of oxidative damage might be a consequence of a higher dilution of reactive oxygen metabolite molecules. Alternatively, the reduction in both haematocrit value and ROM level before egg-laying may be the consequence of the effect of a background variable (possibly a sex hormone). Reduced levels of oxidative damage in breeding females may be caused by the action of oestrogens, which was shown to increase antioxidant enzyme activities (Persky et al. 2000, Viña et al. 2005, Kireev et al. 2007). This change in the level of oxidative damage before egg-laying could be adaptive. Oxidative stress could be deleterious for the production of eggs, as specific proteins, lipids and micronutrients required by the offspring could be damaged (Blount et al. 2016). It was suggested that transition to the reproductive stage triggers a reduction in levels of oxidative damage in order to shield females and in particular their developing eggs from oxidative harm ('oxidative shielding hypothesis'; Blount et al. 2016, Vitikainen et al. 2016, Viblanc et al. 2018). It is noteworthy that a similar decline in ROM level was not found in the treated group, where plasma IGF-1 level increased. High levels of IGF-1 were shown to be related to increased levels of oxidative damage (Holzenberger et al. 2003, Vágási et al. 2020), therefore it is possible that the increased IGF-1 level in the treated group prevented the adaptive preparation for an improved oxidative balance before breeding. However, here we could not demonstrate a correlation of plasma IGF-1 concentration with plasma OXY or ROM levels, thus, other physiological traits may have played important roles in these associations.

A decrease in haematocrit value in reproducing female birds has been commonly observed between pre-breeding and egg-laying (Morton 1994, Williams et al. 2004, Wagner et al. 2008). The reduction in haematocrit value is connected to the elevated blood volume due to osmoregulatory adjustments (i.e. haemodilution) to the increased levels of circulatory yolk precursors (e.g. vitellogenin) (Morton et al. 1994, Williams et al. 2004). High levels of oestrogens could also suppress erythropoiesis, leading to anaemia (Williams et al. 2004, Wagner et al. 2008). We found a significant reduction in haematocrit value in the control group from pre-treatment to pre-laying periods, while in the food-restricted group haematocrit value did not decline. This is the opposite of what was found in several studies, showing that nutritional stress reduced haematocrit value (Boismenu et al. 1992, Merilä and Svensson 1995, Webb 2017). Our study suggests that food-restricted canary females delayed breeding possibly owing to the suppression of hormonal changes in females, causing that haematocrit value showed no decline in that period. Thus, we suggest that the breeding stage of birds should be taken into consideration in interpreting the changes in haematocrit value under stressful conditions.

Female physiological traits (plasma concentration of IGF-1, oxidative stress level and haematocrit value) before egg laying were not related to our measures of reproductive investment (egg mass, yolk mass, eggshell blue-green and brown chroma). Except for eggshell brown colouration, treatment did not influence reproductive investment either (Hargitai et al. 2018). It has been shown that IGF-1 has a positive effect on reproduction in birds (Onagbesan et al. 1999, Kang et al. 2000). For example, the effect of gonadotropins (luteinizing hormone and follicle-stimulating hormone) on follicle development is mediated by intraovarian growth factors, such as IGF-1, which regulate the responsiveness of the ovary to the effect of gonadotropin hormones (Onagbesan and Peddie 1995, Onagbesan et al. 1999). Thus, we hypothesised that females with higher circulating IGF-1 levels before laying would invest more in the egg macro- and micronutrient contents, but we failed to find such results. Lodjak et al. (2018) reported that in passerines there was a negative association between plasma IGF-1 level and egg weight in species with smaller body size and shorter duration of parental care, while the association was the opposite in bird species with larger body size and longer duration of parental care, indicating that the effect of IGF-1 on specific reproductive traits could be plastic. It is possible that IGF-1 level influenced other, unstudied components of the reproductive investment in the canary.

In some bird species, positive associations were reported between female antioxidant status and egg traits. For

example, in the brown booby *Sula leucogaster*, females with higher level of oxidative stress before egg-laying laid smaller eggs (Montoya et al. 2016). In the blue-footed booby *Sula nebouxii* and the mallard *Anas platyrhynchos*, antioxidant-supplemented females laid eggs with more intense blue colouration (Morales et al. 2011, Butler and McGraw 2013). Moreover, a positive association was reported between female antioxidant capacity and eggshell blue colour intensity in the grey catbird *Dumetella caroliensis* (Hanley et al. 2008). Our previous study reported that antioxidant-supplemented canary females laid eggs with more intense blue-green colouration (Hargitai et al. 2016). However, here we could not find support for our hypothesis that the female's plasma level of oxidative status would be related to egg traits, as female OXY and ROM levels before egg-laying were not related either to clutch mean egg mass, yolk mass or eggshell colouration in the canary.

Conclusions

Our results showed that food restriction delayed egg-laying in canaries, and also caused changes in plasma physiological traits (plasma IGF-1 and ROM levels, haematocrit value) at the pre-laying period. Our results suggest that nutritional limitation caused a shift in the timing of egg-laying that could be mediated by concomitant changes in physiological variables, therefore our study highlights the importance of breeding stage when studying and interpreting the effects of food restriction on physiological traits of breeding birds. However, we did not find support for the hypothesis that plasma physiological traits at the pre-laying period would influence investment in egg traits in the canary. Further research may focus on whether investment in egg traits during stressful conditions exerts a cost of reproduction on female birds.

Funding – This study was supported by the Hungarian Scientific Research Fund (OTKA, grants no. PD100304 to RH and no. K139021 to ÁZL) and the Bolyai János Research Fellowship (MTA) to RH. We are grateful for the facilities and equipment provided by the Department of Systematic Zoology and Ecology of Eötvös Loránd University. During manuscript preparation ZT and ÁZL were supported by the European Union and the European Social Fund (EFOP-3.6.1-16-2016-00022).
Permissions – The National Food Chain Safety Office (NÉBIH) provided permissions for this study (PEI/001/824-4/2015). All applicable international, national and institutional guidelines for the care and use of animals were followed.

Author contributions

Rita Hargitai: Conceptualization (lead); Formal analysis (lead); Funding acquisition (lead); Investigation (equal); Methodology (lead); Resources (lead); Visualization (lead); Writing – original draft (lead); Writing – review and editing (equal). **Nóra Boross**: Investigation (supporting); Writing – review and editing (supporting). **Zsófia Tóth**: Conceptualization (supporting); Investigation (supporting); Methodology (supporting); Writing – review and editing (equal). **Ádám Z. Lendvai**: Funding acquisition (supporting); Resources (supporting); Writing – review and editing (equal).

Transparent Peer Review

The peer review history for this article is available at <https://publons.com/publon/10.1111/jav.02866>.

Data availability statement

Data are available from the Dryad Digital Repository: <https://doi.org/10.5061/dryad.0cfxpnw47> (Hargitai et al. 2022).

Supporting information

The supporting information associated with this article is available from the online version.

References

Afonso, S., Vanore, G. and Batlle, A. 1999. Protoporphyrin IX and oxidative stress. – Free Rad. Res. 31: 161–170.

Alan, R. R. and McWilliams, S. R. 2013. Oxidative stress, circulating antioxidants and dietary preferences in songbirds. – Comp. Biochem. Physiol. B 164: 185–193.

Ayson, F. G., de Jesus-Ayson, E. G. T. and Takemura, A. 2007. mRNA expression patterns for GH, PRL, SL, IGF-I and IGF-II during altered feeding status in rabbitfish, *Siganus guttatus*. – Gen. Comp. Endocrinol. 150: 196–204.

Baker, J., Liu, J. P., Robertson, E. J. and Efstratiadis, A. 1993. Role of insulin-like growth factors in embryonic and postnatal growth. – Cell 75: 73–82.

Barja, G. 2002. Endogenous oxidative stress: relationship to aging, longevity and caloric restriction. – Ageing Res. Rev. 1: 397–411.

Blount, J. D., Vitikainen, E. I., Stott, I. and Cant, M. A. 2016. Oxidative shielding and the cost of reproduction. – Biol. Rev. 91: 483–497.

Boismenu, C., Gauthier, G. and Larochelle, J. 1992. Physiology of prolonged fasting in greater snow geese *Chen caerulescens atlantica*. – Auk 109: 511–521.

Butler, M. W. and McGraw, K. J. 2013. Eggshell colouration reflects both yolk characteristics and dietary carotenoid history of female mallards. – Funct. Ecol. 27: 1176–1185.

Buyse, J., Decuypere, E., Darras, V. M., Vleurick, L. M., Kuhn, E. R. and Veldhuis, J. D. 2000. Food deprivation and feeding of broiler chickens is associated with rapid and interdependent changes in the somatotrophic and thyrotrophic axes. – Br. Poult. Sci. 41: 107–116.

Catoni, C., Peters, A. and Schaefer, H. M. 2008. Life history trade-offs are influenced by the diversity, availability and interactions of dietary antioxidants. – Anim. Behav. 76: 1107–1119.

Cohen, A. A., McGraw, K. J. and Robinson, W. D. 2009. Serum antioxidant levels in wild birds vary in relation to diet, season, life history strategy and species. – Oecologia 161: 673–683.

Costantini, D. 2016. Oxidative stress ecology and the d-ROMs test: facts, misfacts and an appraisal of a decade's work. – Behav. Ecol. Sociobiol. 70: 809–820.

Costantini, D., Casasole, G., AbdElgawad, H., Asard, H. and Eens, M. 2016. Experimental evidence that oxidative stress influences reproductive decisions. – Funct. Ecol. 30: 1169–1174.

Daftary, S. S. and Gore, A. C. 2005. IGF-1 in the brain as a regulator of reproductive neuroendocrine function. – Exp. Biol. Med. 230: 292–306.

Dantzer, B. and Swanson, E. M. 2012. Mediation of vertebrate life histories via insulin-like growth factor-1. – Biol. Rev. 87: 414–429.

Dawson, A. 1983. Plasma gonadal steroid levels in wild starlings *Sturnus vulgaris* during the annual cycle and in relation to the stages of breeding. – Gen. Comp. Endocrinol. 49: 286–294.

du Dot, T. J., Rosen, D. A., Richmond, J. P., Kitaysky, A. S., Zinn, S. A. and Trites, A. W. 2009. Changes in glucocorticoids, IGF-I and thyroid hormones as indicators of nutritional stress and subsequent refeeding in Steller sea lions *Eumetopias jubatus*. – Comp. Biochem. Physiol. 152: 524–534.

Eitan, Y., Soller, M. and Rozenboim, I. 1998. Comb size and estrogen levels toward the onset of lay in broiler and layer strain females under ad libitum and restricted feeding. – Poult. Sci. 77: 1593–1600.

Feoli, A. M., Siqueira, I. R., Almeida, L., Tramontina, A. C., Vanzella, C., Sbaraini, S., Schweigert, I. D., Netto, C. A., Perry, M. L. and Gonçalves, C. A. 2006. Effects of protein malnutrition on oxidative status in rat brain. – Nutrition 22: 160–165.

Finkel, T. and Holbrook, N. J. 2000. Oxidants, oxidative stress and the biology of ageing. – Nature 408: 239–247.

Fletcher, Q. E., Selman, C., Boutin, S., McAdam, A. G., Woods, S. B., Seo, A. Y., Leeuwenburgh, C., Speakman, J. R. and Humphries, M. M. 2013. Oxidative damage increases with reproductive energy expenditure and is reduced by food-supplementation. – Evolution 67: 1527–1536.

Giordano, M., Costantini, D., Pick, J. L. and Tschirren, B. 2015. Female oxidative status, egg antioxidant protection and eggshell pigmentation: a supplemental feeding experiment in great tits. – Behav. Ecol. Sociobiol. 69: 777–785.

Halliwell, B. and Gutteridge, J. M. C. 2007. Antioxidant defences: endogenous and diet derived. – Free Rad. Biol. Med. 4: 79–186.

Hanley, D., Heiber, G. and Dearborn, D. C. 2008. Testing an assumption of the sexual-signaling hypothesis: does blue-green egg colour reflect maternal antioxidant capacity. – Condor 110: 767–771.

Hargitai, R., Boross, N., Nyiri, Z. and Eke, Z. 2016. Biliverdin- and protoporphyrin-based eggshell pigmentation in relation to antioxidant supplementation, female characteristics and egg traits in the canary *Serinus canaria*. – Behav. Ecol. Sociobiol. 70: 2093–2110.

Hargitai, R., Boross, N., Nyiri, Z. and Eke, Z. 2018. Effects of food limitation on the intensity of blue-green and brown eggshell colouration: an experimental study with the canary. – J. Avian Biol. 49: jav-01486.

Hargitai, R., Boross, N., Tóth, Z. and Lendvai, Á. Z. 2022. Data from: Food restriction delays breeding and affects insulin-like growth factor-1, oxidative damage and haematocrit value before egg-laying in female canaries. – Dryad Digital Repository, <https://doi.org/10.5061/dryad.0cfxpnw47>.

Harvey, S., Klandorf, H. and Phillips, J. G. 1981. Effect of food or water deprivation on circulating levels of pituitary, thyroid and adrenal hormones and on glucose and electrolyte concentrations in domestic ducks *Anas platyrhynchos*. – J. Zool. 194: 341–361.

Helle, S. I., Omsjø, I. H., Cwyfan Hughes, S. C., Botta, L., Hüls, G., Holly, J. M. P. and Lønning, P. E. 1996. Effects of oral and transdermal oestrogen replacement therapy on plasma levels of insulin-like growth factors and IGF binding proteins 1 and 3: a cross-over study. – Clin. Endocrinol. 45: 727–732.

Ho, K. K., O'Sullivan, A. J., Weissberger, A. J. and Kelly, J. J. 1996. Sex steroid regulation of growth hormone secretion and action. – Horm. Res. Paediatr. 45: 67–73.

Hocking, P. M., Bernard, R., Wilkie, R. S. and Goddard, C. 1994. Plasma growth hormone and insulin-like growth factor-I (IGF-I) concentrations at the onset of lay in ad libitum and restricted broiler breeder fowl. – Br. Poult. Sci. 35: 299–308.

Holzenberger, M., Dupont, J., Ducos, B., Leneuve, P., Géloën, A., Even, P. C., Pascale, C. and Le Bouc, Y. 2003. IGF-1 receptor regulates lifespan and resistance to oxidative stress in mice. – Nature 421: 182–187.

Kang, W. J., Yun, J. S., Seo, D. S., Lee, C. Y., Hong, K. C. and Ko, Y. 2000. Study on the association of early egg productivity with serum insulin-like growth factor-I in Korean Native Ogol fowls. – Korean J. Anim. Sci. 42: 767–776.

Kaur, H., Hughes, M. N., Green, C. J., Naughton, P., Foresti, R. and Motterlini, R. 2003. Interaction of bilirubin and biliverdin with reactive nitrogen species. – FEBS Lett. 543: 113–119.

Kennedy, G. Y. and Vevers, H. G. 1976. A survey of avian eggshell pigments. – Comp. Biochem. Physiol. B 55: 117–123.

Kim, M. H., Seo, D. S. and Ko, Y. 2004. Relationship between egg productivity and insulin-like growth factor-I genotypes in Korean native Ogol chickens. – Poult. Sci. 83: 1203–1208.

Kireev, R. A., Tresguerres, A. F., Vara, E., Ariznavarreta, C. and Tresguerres, J. A. F. 2007. Effect of chronic treatments with GH, melatonin, estrogens and phytoestrogens on oxidative stress parameters in liver from aged female rats. – Biogerontology 8: 469–482.

König, K. and Meyer, H. 1992. Photodynamically induced inactivation of propionibacterium acnes using the photosensitizer methylene blue and red light. – Zentralb. Gynaekol. 178: 297–300.

Lauterio, T. J. and Scanes, C. G. 1987. Hormonal responses to protein restriction in two strains of chickens with different growth characteristics. – J. Nutr. 117: 758–763.

Lendvai, Á. Z., Tóth, Z., Mahr, K., Osváth, G., Vogel-Kindgen, S. and Gander, B. A. 2021. Effects of experimental increase in insulin-like growth factor 1 on feather growth rate, moult intensity and feather quality in a passerine bird. – J. Exp. Biol. 224: jeb242481.

Lodjak, J. and Verhulst, S. 2020. Insulin-like growth factor 1 of wild vertebrates in a life-history context. – Mol. Cell. Endocrinol. 518: 110978.

Lodjak, J., Mägi, M. and Tilgar, V. 2014. Insulin-like growth factor 1 and growth rate in nestlings of a wild passerine bird. – Funct. Ecol. 28: 159–166.

Lodjak, J., Mägi, M., Sild, E. and Mänd, R. 2017. Causal link between insulin-like growth factor 1 and growth in nestlings of a wild passerine bird. – Funct. Ecol. 31: 184–191.

Lodjak, J., Mänd, R. and Mägi, M. 2018. Insulin-like growth factor 1 and life-history evolution of passerine birds. – Funct. Ecol. 32: 313–323.

Lodjak, J., Tilgar, V. and Mägi, M. 2016. Does the interaction between glucocorticoids and insulin-like growth factor 1 predict nestling fitness in a wild passerine? – Gen. Comp. Endocrinol. 225: 149–154.

Mahr, K., Vincze, O., Tóth, Z., Hoi, H. and Lendvai, Á. Z. 2020. Insulin-like growth factor 1 is related to the expression of plumage traits in a passerine species. – Behav. Ecol. Sociobiol. 74: 1–12.

Malarkey, W. B., Burleson, M., Cacioppo, J. T., Poehlmann, K., Glaser, R. and Kiecolt-Glaser, J. K. 1997. Differential effects of estrogen and medroxyprogesterone on basal and stress-induced growth hormone release, IGF-1 levels and cellular immunity in postmenopausal women. – Endocrine 7: 227–233.

McDonagh, A. F. 2001. Turning green to gold. – Nat. Struct. Biol. 8: 198–200.

McMurtry, J. P., Francis, G. L. and Upton, Z. 1997. Insulin-like growth factors in poultry. – Dom. Anim. Endocrinol. 14: 199–229.

Meijer, T., Langer, U. 1995. Food availability and egg-laying of captive European starlings. – Condor 97: 718–728.

Merilä, J. and Svensson, E. 1995. Fat reserves and health state in migrant goldcrest *Regulus regulus*. – Funct. Ecol. 9: 842–848.

Mikšík, I., Holáň, V. and Deyl, Z. 1996. Avian eggshell pigments and their variability. – Comp. Biochem. Physiol. B 113: 607–612.

Monaghan, P., Metcalfe, N. B. and Torres, R. 2009. Oxidative stress as a mediator of life history tradeoffs: mechanisms, measurements and interpretation. – Ecol. Lett. 12: 75–92.

Montoya, B., Valverde, M., Rojas, E. and Torres, R. 2016. Oxidative stress during courtship affects male and female reproductive effort differentially in a wild bird with biparental care. – J. Exp. Biol. 219: 3915–3926.

Morales, J., Velando, A. and Torres, R. 2011. Biliverdin-based egg colouration is enhanced by carotenoid supplementation. – Behav. Ecol. Sociobiol. 65: 197–203.

Morton, M. L. 1994. Hematocrits in montane sparrows in relation to reproductive schedule. – Condor 96: 119–126.

Onagbesan, O. M. and Peddie, M. J. 1995. Effects of insulin-like growth factor I and interactions with transforming growth factor α and LH on proliferation of chicken granulosa cells and production of progesterone in culture. – Reproduction 104: 259–265.

Onagbesan, O. M., Metayer, S., Tona, K., Williams, J., Decuypere, E. and Bruggeman, V. 2006. Effects of genotype and feed allowance on plasma luteinizing hormones, follicle-stimulating hormones, progesterone, estradiol levels, follicle differentiation and egg production rates of broiler breeder hens. – Poult. Sci. 85: 1245–1258.

Onagbesan, O. M., Vleugels, B., Buys, N., Bruggeman, V., Safi, M. and Decuypere, E. 1999. Insulin-like growth factors in the regulation of avian ovarian functions. – Dom. Anim. Endocrinol. 17: 299–313.

Persky, A. M., Greene, P. S., Stubley, L., Howell, C. O., Zaulyanov, L., Brazeau, G. A. and Simpkins, J. W. 2000. Protective effect of estrogens against oxidative damage to heart and skeletal muscle in vivo and in vitro. – Proc. Soc. Exp. Biol. Med. 223: 59–66.

Pitetti, J. L., Calvel, P., Zimmermann, C., Conne, B., Papaioannou, M. D., Aubry, F., Cederroth, C. R., Umer, F., Fumel, B., Crausaz, M., Docquier, M., Herrera, P. L., Pralong, F., Germond, M., Guillou, F., Jégou, B. and Nef, S. 2013. An essential role for insulin and IGF1 receptors in regulating sertoli cell proliferation, testis size and FSH action in mice. – Mol. Endocrinol. 27: 814–827.

Rasmussen, M. H., Frystyk, J., Andersen, T., Breum, L., Christiansen, J. S. and Hilsted, J. 1994. The impact of obesity, fat distribution and energy restriction on insulin-like growth factor-1 (IGF-1), IGF-binding protein-3, insulin and growth hormone. – Metabolism 43: 315–319.

Ricklefs, R. E. and Wikelski, M. 2002. The physiology/lifehistory nexus. – Trends Ecol. Evol. 17: 462–468.

Singleton, J. W. and Laster, L. 1965. Biliverdin reductase of guinea pig liver. – J. Biol. Chem. 240: 4780–4789.

Sohal, R. S., Ku, H. H., Agarwal, S., Forster, M. J. and Lal, H. 1994. Oxidative damage, mitochondrial oxidant generation and antioxidant defenses during aging and in response to food restriction in the mouse. – Mech. Ageing Dev. 74: 121–133.

Sparkman, A. M., Byars, D., Ford, N. B. and Bronikowski, A. M. 2010. The role of insulin-like growth factor-1 (IGF-1) in growth and reproduction in female brown house snakes *Lamprophis fuliginosus*. – Gen. Comp. Endocrinol. 168: 408–414.

Sparkman, A. M., Vleck, C. M. and Bronikowski, A. M. 2009. Evolutionary ecology of endocrine-mediated life-history variation in the garter snake *Thamnophis elegans*. – Ecology 90: 720–728.

Speakman, J. R. and Selman, C. 2011. The free-radical damage theory: accumulating evidence against a simple link of oxidative stress to ageing and lifespan. – Bioessays 33: 255–259.

Stearns, S. C. 1989. Trade-offs in life-history evolution. – Funct. Ecol. 3: 259–268.

Surai, P. F. 2002. Natural antioxidants in avian nutrition and reproduction. – Nottingham Univ. Press.

Swanson, E. M. and Dantzer, B. 2014. Insulin-like growth factor-1 is associated with life-history variation across Mammalia. – Proc. R. Soc. B 281: 20132458.

Swennen, Q., Geraert, P. A., Mercier, Y., Everaert, N., Stinckens, A., Willemsen, H., Li, Y., Decuypere, E. and Buyse, J. 2011. Effects of dietary protein content and 2-hydroxy-4-methylthiobutanoic acid or DL-methionine supplementation on performance and oxidative status of broiler chickens. – Br. J. Nutr. 106: 1845–1854.

Totzke, U., Fenske, M., Hüppop, O., Raabe, H. and Schach, N. 1999. The influence of fasting on blood and plasma composition of herring gulls *Larus argentatus*. – Phys. Biochem. Zool. 72: 426–437.

Vágási, C. I., Tóth, Z., Pénzes, J., Pap, P. L., Ouyang, J. Q. and Lendvai, Á. Z. 2020. The relationship between hormones, glucose and oxidative damage is condition and stress dependent in a free-living passerine bird. – Physiol. Biochem. Zool. 93: 466–476.

Van Heemst, D. 2010. Insulin, IGF-1 and longevity. – Aging Dis. 1: 147.

Viblanc, V. A., Schull, Q., Rabdeau, J., Roth, J. D., Dobson, F. S., Criscuolo, F., Ulrich, P. and Saraux, C. 2018. Maternal oxidative stress and reproduction: testing the constraint, cost and shielding hypotheses in a wild mammal. – Funct. Ecol. 32: 722–735.

Viña, J., Borrás, C., Gambini, J., Sastre, J. and Pallardó, F. V. 2005. Why females live longer than males? Importance of the upregulation of longevity-associated genes by oestrogenic compounds. – FEBS Lett. 579: 2541–2545.

Vitikainen, E. I., Cant, M. A., Sanderson, J. L., Mitchell, C., Nichols, H. J., Marshall, H. H., Thompson, F. J., Gilchrist, J. S., Hodge, S. J., Johnstone, R. A. and Blount, J. D. 2016. Evidence of oxidative shielding of offspring in a wild mammal. – Front. Ecol. Evol. 4: 58.

Wagner, E. C., Stables, C. A. and Williams, T. D. 2008. Hematological changes associated with egg production: direct evidence for changes in erythropoiesis but a lack of resource dependence?. – J. Exp. Biol. 211: 2960–2968.

Webb, A. C., Chick, L. D., Cobb, V. A. and Klukowski, M. 2017. Effects of moderate food deprivation on plasma corticosterone and blood metabolites in common watersnakes *Nerodia sipedon*. – J. Herpetol. 51: 134–141.

Weber, G. M. and Sullivan, C. V. 2000. Effects of insulin-like growth factor-I on in vitro final oocyte maturation and ovarian steroidogenesis in striped bass, *Morone saxatilis*. – Biol. Reprod. 63: 1049–1057.

Williams, T. D., Challenger, W. O., Christians, J. K., Evanson, M., Love, O. and Vezina, F. 2004. What causes the decrease in haematocrit during egg production? – Funct. Ecol. 18: 330–336.

Yu, B. P. 1996. Aging and oxidative stress: modulation by dietary restriction. – Free Rad. Biol. Med. 21: 651–668.

Zera, A. J. and Harshman, L. G. 2001. The physiology of life history trade-offs in animals. – Annu. Rev. Ecol. Syst. 32: 95–126.

Zulu, V. C., Nakao, T. and Sawamukai, Y. 2002. Insulin-like growth factor-I as a possible hormonal mediator of nutritional regulation of reproduction in cattle. – J. Vet. Med. Sci. 64: 657–665.