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21 **Fat provisioning in winter impairs egg production during the**
22 **following spring: a landscape-scale study of blue tits**

23

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40 **Running title:** Winter provisioning effects on egg production

41 **SUMMARY**

42 **1.** Provisioning of garden birds is a growing phenomenon, particularly during winter, but
43 there is little empirical evidence of its true ecological impacts. One possibility is that winter
44 provisioning could enhance subsequent breeding performance, but this seems likely to depend
45 on the types of nutrients provided. For example, whereas effects of macronutrients such as fat
46 are unlikely to be carried over to influence breeding in small passerines, micronutrients such
47 as dietary vitamin E (an antioxidant) may be stored or have lasting health benefits.

48 **2.** Here, we examine the carry-over effects of winter food supplements on egg production in
49 wild populations of blue tits (*Cyanistes caeruleus*). Over three consecutive years birds were
50 provisioned with fat, fat-plus-vitamin E, or remained unfed (controls).

51 **3.** The provision of fat in winter resulted in smaller relative yolk mass in larger eggs, and
52 reduced yolk carotenoid concentrations in early breeders. However these effects were not
53 seen in birds provisioned with fat-plus-vitamin E. Lay date, clutch size, egg mass and yolk
54 vitamin E concentrations were not significantly affected by winter provisioning treatment.

55 **4.** Our results indicate that winter provisioning can have important downstream
56 consequences, in particular affecting investment in egg production several weeks or months
57 later.

58 **5.** Provisioning is widely applied to support garden bird populations, and for the
59 conservation management of endangered species. However, our results challenge the
60 assumption that such practices are always beneficial at the population level, and emphasise
61 how the ecological impacts can depend on the specific nutritional profile of provisioned
62 foods.

63 **Key words:** Antioxidant; carry-over effect; egg quality; life-history trade-off; maternal effect;
64 supplementary feeding; vitamin E

65 INTRODUCTION

66 The evolution of life-history traits is constrained by the existence of trade-offs amongst them;
67 at the proximate level such trade-offs are often modulated by variation in the supply of dietary
68 resources (Stearns 1992). Indeed, food supply plays a crucial role in avian ecology, by
69 influencing community structure, regulating population sizes and through effects on
70 individual behaviours and life-histories (Newton 1998). For birds in temperate climates,
71 access to sufficient dietary resources is likely to vary seasonally, leading Lack (1954)
72 to hypothesise that populations would be regulated by food availability during the period of
73 least abundance; the ‘winter food limitation hypothesis’. Consequently, over-winter food
74 supply has been shown to enhance body condition and survival in the short-term (Jansson,
75 Ekman & Vonbromssen 1981; Brittingham & Temple 1988; Grubb & Cimprich 1990). This
76 is of particular relevance to the *ad hoc* supplementation of foods within urban habitats, a
77 hugely popular and growing phenomenon worldwide (Jones & Reynolds 2008). Provisioning
78 of garden birds is most prevalent in winter months, when energetic demands are high and
79 natural food is relatively scarce (Chamberlain *et al.* 2005). Indeed, the UK and US together
80 purchase in excess of 500,000 tonnes of commercial bird food each year (O’Leary & Jones
81 2006), but the ecological consequences of this enormous resource are little studied (Jones &
82 Reynolds 2008; Robb *et al.* 2008a).

83

84 Whilst the capacity for winter food availability to affect survival is well established, there can
85 be other, downstream consequences that remain poorly understood. Carry-over effects arise
86 when events in one season or year influence an individual’s performance in a later season or
87 year (Harrison *et al.* 2011). The potential for natural food availability on the wintering
88 grounds to influence subsequent productivity in migratory birds is well known (reviewed by
89 Harrison *et al.* 2011). However while numerous experimental studies have investigated the

90 effects of food supplementation just prior to or during breeding on productivity in wild birds
91 (e.g. Svensson & Nilsson 1995; Blount *et al.* 2002b), effects across seasons have rarely been
92 quantified (Park, Lee & Rhim 2004; Robb *et al.* 2008b). In a landscape-scale investigation,
93 Robb *et al.* (2008b) found that blue tits provisioned with peanuts during winter had advanced
94 laying dates and increased fledging success compared to unfed controls, even though
95 provisioning had stopped 6 weeks prior to breeding.

96

97 The mechanisms that underlie such carry-over effects of winter provisioning on garden birds
98 are poorly understood. Until recently, it has been assumed that carry-over effects are largely
99 driven by energy availability. However, as ‘income breeders’, small passerines cannot store
100 macronutrients such as fat to any great extent and must rely on daily food intake to meet the
101 energetic demands of reproduction (Drent & Daan 1980). It has recently been suggested,
102 however, that any micronutrient with the potential to be stored could produce carry-over
103 effects (Harrison *et al.* 2011). In particular, large amounts of fat-soluble dietary antioxidants
104 such as vitamin E and carotenoids can accumulate in subcutaneous fat and liver in birds,
105 forming reserves which may be drawn upon during the breeding season when demand is
106 increased (Negro *et al.* 2001; Surai 2007; Metzger & Bairlein 2011). Antioxidants can have
107 important effects on life histories. For example, vitamin E and carotenoids provide
108 antioxidant defence against reactive oxygen species (ROS) which result as by-products of
109 metabolism (reviewed by Catoni, Peters & Schaefer 2008; Monaghan, Metcalfe & Torres
110 2009). In doing so, they prevent important biomolecules from being damaged by ROS (i.e.
111 oxidative stress), which might otherwise lead to physiological dysfunction, disease
112 progression and aging. However, we are not aware of any study which has tested whether
113 winter provisioning with an antioxidant may have carry-over effects on reproduction in any
114 species.

115

116 Whilst it is generally assumed that provisioning of food to wild birds is beneficial at the
117 individual- and population levels, in fact this is far from clear (Robb *et al.* 2008a; Harrison *et*
118 *al.* 2010). For example, although winter provisioning may enhance short-term survival, it
119 could result in dependency on feeders, reduced dietary diversity and could even have direct
120 deleterious effects on health (Jones & Reynolds 2008). For example, studies of laboratory
121 rodents have shown that increased dietary carbohydrate and fatty acid availability up-
122 regulates mitochondrial respiration (Iossa *et al.* 2002), which can result in increased
123 susceptibility to oxidative stress (e.g. Igosheva *et al.* 2010). Therefore, it is conceivable that
124 winter provisioning of birds could have negative downstream effects on reproductive
125 capacity, but this remains to be experimentally tested. The potential for such deleterious
126 effects seems likely to depend on the nutritional composition of foods. For example, winter
127 provisioning with macronutrients such as fats could in theory impair subsequent reproduction,
128 whereas dietary antioxidants may mitigate any such deleterious effects of macronutrient
129 metabolism.

130

131 One aspect of reproduction which seems especially likely to be influenced by winter
132 provisioning is egg production. In birds, egg production carries high costs both in terms of
133 energy and nutrient requirements (Perrins 1996). The egg's lipid- and protein-rich yolk
134 provides the main source of energy for developing embryos and variation in yolk mass can
135 influence offspring survival (Williams 1994). Nevertheless, it appears that energy supply *per*
136 *se* is rarely limiting for egg production (e.g. Bolton, Houston & Monaghan 1992). The yolk
137 also contains maternally-derived antioxidants including carotenoids and vitamin E, deposition
138 of which is thought to comprise a maternal effect to enhance offspring performance
139 (McGraw, Adkins-Regan & Parker 2005). The availability of diet-derived antioxidants is

140 potentially limiting for egg production due to scarcity of antioxidants in the environment
141 and/or physiological trade-offs in their usage (Blount, Houston & Møller 2000). Increased
142 deposition of carotenoids into yolk, resulting from supplemental feeding just prior to and
143 during laying, has been shown to result in reduced yolk susceptibility to lipid peroxidation
144 (Blount *et al.* 2002a; McGraw 2005), and enhanced hatching success (Møller, Karadas &
145 Mousseau 2008), immunity (Saino *et al.* 2003), nestling plumage colouration (Biard, Surai &
146 Møller 2005), and adult survival and sexual signal expression (McGraw, Adkins-Regan &
147 Parker 2005). There is also considerable evidence that yolk-derived vitamin E can function as
148 a potent antioxidant and immunostimulant *in vivo* (reviewed by Surai 2007). However, few
149 studies have investigated whether limitations in the supply of vitamin E may underlie life
150 history trade-offs (but see de Ayala, Martinelli & Saino 2006). Remarkably, there have been
151 no studies of the effects of vitamin E availability on egg production in wild birds, and whether
152 a female's capacity to produce high quality eggs is affected by her access to dietary
153 antioxidants in the preceding winter awaits study.

154

155 Any effects of winter provisioning seem likely to be context-dependent, being affected by
156 habitat quality and natural food availability (Kallander 1981). Therefore it is important to
157 study the ecological consequences of dietary provisioning at a landscape scale, across
158 multiple sites and years (Robb *et al.* 2008a). Here, we use such an approach to examine the
159 effects of winter provisioning of wild blue tits on egg production during the following spring.
160 To investigate the roles of energy and antioxidants as possible mediators of carry-over effects,
161 populations were fed either fat, fat-plus-vitamin E or remained unfed (controls). Our aim was
162 to determine whether winter provisioning altered the timing of laying, clutch size, or egg
163 quality in terms of the relative mass of egg components, and levels of yolk antioxidants
164 (vitamin E and carotenoids). We hypothesised that compared to unfed controls, winter

165 provisioning with fat would have no beneficial effects on egg production, whereas winter
166 provisioning with fat-plus-vitamin E would enhance egg production. Alternatively, if winter
167 provisioning with a macronutrient such as fat impairs future egg production compared to
168 unfed controls, we hypothesised that this impairment would not be seen in birds provisioned
169 with fat-plus-vitamin E.

170 **MATERIALS AND METHODS**

171 **Study site and experimental design**

172 The winter provisioning experiment was conducted over three years from 2007 to 2009 and
173 carry-over effects were measured during the subsequent breeding seasons, 2008 – 2010
174 respectively. The study took place in Cornwall, UK, at nine deciduous woodland sites, where
175 oak (*Quercus* spp.), beech (*Fagus sylvatica*), sweet chestnut (*Castanea sativa*) and sycamore
176 (*Acer pseudoplatanus*) were the predominant tree species. Sites averaged 10.7 hectares in
177 size and were situated at least 2km apart to minimise the possibility of between-site
178 movements of birds. There was no evidence from adult ringing records that birds moved sites
179 between winter and spring, or from year to year. Sites were nominally grouped into three
180 triplets, according to similarities in the composition of tree, understory and ground cover
181 species. Woodland size, proximity to settlements, level of public access and amount of
182 periphery woodland were also taken into account.

183

184 In the first year, each site within a triplet was randomly allocated to one of three provisioning
185 groups: (1) no supplement (hereafter ‘control’), (2) fat only (hereafter ‘fat’) and (3) fat-plus-
186 vitamin E (hereafter ‘fat+VE’). Treatments were rotated within triplets across years, so that
187 every site received all three treatments over the course of the study. Since treatments were

188 replicated three times using different triplet groups in a given year, any potential confounding
189 effects of year have been avoided.

190

191 Within the six fed sites each year, feeders were hung *ca.* 4m from the ground at 100m
192 intervals along parallel transects (100m apart) and at an average of nine per site. Feeders were
193 custom-designed to prevent access by grey squirrels (*Sciurus carolinensis*) and other
194 mammals and larger bird species. A total of 346 nest boxes, with a 32mm diameter entrance
195 hole, were positioned across all sites (mean \pm SE per site: 38 ± 1.4). Boxes were erected at
196 25m intervals along transects and parallel to feeders, such that each box was no more than
197 50m from a feeder. This design produced an equal density and distribution of feeders and
198 boxes across individual woodlands, *ca.* one feeder and four boxes per hectare.

199 **Winter provisioning experiment**

200 Food was provisioned through the winter only (14 Dec – 4 Mar 2007/08; 18 Nov – 11 Mar
201 2008/09 and 2009/10), leaving a gap of at least one month before laying commenced (8 Apr,
202 11 Apr, 15 Apr respectively) and thus allowing carry-over effects to be investigated with
203 confidence. All feeding stations were provisioned with a fresh 150g fat ball every 10 days.
204 Fat balls for the fat+VE treatment group were supplemented with α -tocopherol (T3251;
205 Sigma-Aldrich, Dorset, UK) at a concentration of 100 mg kg⁻¹fat, a level equivalent to that
206 found in peanuts (Chun, Lee & Eitenmiller 2005), a popular food provisioned to garden birds.
207 α -Tocopherol cannot be provisioned to wild birds without the use of a ‘carrier’ and, as a
208 lipophilic molecule, it is inevitably co-acquired with fat in natural foods (Blount *et al.* 2002b).
209 Therefore, the fat+VE treatment group provides an ecologically realistic test of the effects of
210 antioxidant provisioning.

211

212 All fat balls were produced from solid vegetable fat (Crisp 'n Dry, Princes Ltd., Liverpool,
213 UK) 1 – 2 days in advance of provisioning, using standardised methods adapted from Blount
214 et al. (2002b). Fat was heated to 60°C for *ca.* 1 hour until liquefied, then cooled on ice until
215 viscous. When the fat reached 18 – 20°C, yellow food colouring (0.125ml/ 100g fat; ASDA
216 Natural Food Colouring, Asda Stores Ltd., Leeds) was added, to increase fat ball
217 attractiveness to target species (McGraw *et al.* 2006) verified by a pilot study (unpubl. data).
218 At the same time α -Tocopherol was added to fat (fat+VE treatment) at the concentration
219 specified above and stirred thoroughly for 4 mins to homogenise. Fat balls (150g) were
220 hardened at -20°C overnight before being deployed. Upon collection fat balls were weighed
221 (± 0.01 g) to determine levels of consumption. Observations at feeders, beak markings on fat
222 balls and winter mist netting confirmed food use was dominated by Parid sp., with a mean of
223 3.32 kg (± 0.12 SE) consumed per site per year. Ring recoveries and stable isotope analysis
224 provided evidence that winter provisioned foods had been utilised by breeding birds
225 (Plummer 2011). Food uptake per 10 day feeding period differed between years, but was not
226 significantly different between treatment groups (general linear mixed model [GLMM] with
227 site/ feeder random factor; treatment: $\chi^2_1 = 0.36$, $p = 0.55$, year: $\chi^2_2 = 336.29$, $p < 0.001$,
228 treatment \times year: $\chi^2_2 = 0.03$, $p = 0.98$).

229 **Breeding parameters**

230 Nest boxes were inspected every 1 – 3 days from April to June. Lay date of the first egg was
231 back-calculated by assuming one egg was laid per day, if more than one egg was present
232 (Perrins 1996). After the first egg was laid, nests were visited every 1 – 2 days until clutch
233 completion and new eggs were marked to establish laying order. Total clutch size was
234 recorded upon clutch completion and total clutch mass determined (± 0.1 g) using an electronic
235 balance. One egg, typically the last- or second-to-last egg laid, was then removed for

236 measurement of mass and biochemical analysis. Collected eggs were returned to the
237 laboratory, weighed ($\pm 0.001\text{g}$) and dissected on the day of collection. The yolk was rolled
238 over damp filter paper to remove traces of albumen, weighed ($\pm 0.001\text{g}$) and stored at -80°C
239 until analysis.

240 **Biochemical assays**

241 For extraction of antioxidants, egg yolk (0.040-0.050g) was vortexed in 0.7mL 5% NaCl for 5
242 seconds and then homogenised with 1mL EtOH for 20 sec. Hexane (1.5mL) was added and
243 samples were further homogenised for 10 sec, before being centrifuged for 4 minutes at 8000
244 \times g and the hexane phase containing the antioxidants drawn off. Extraction was repeated and
245 both hexane extracts combined.

246

247 Total carotenoid concentrations in egg yolk were determined by spectrophotometry at
248 450nm (Nicolet Evolution 500; Thermo Electron Corp., Hemel Hemstead, U.K.) with total
249 carotenoid concentration calculated using the extinction coefficient of lutein in hexane (2589,
250 Craft & Soares 1992). Hexane (500 μl) was evaporated to dryness and the residue re-
251 dissolved in 150 μl DCM and 150 μl MeOH. For determination of α -tocopherol
252 concentrations, samples (20 μl) were injected into a high-performance liquid chromatography
253 system (HPLC; Dionex Corporation, California, USA). Separation utilised a 3 μ C₁₈ reverse-
254 phase column (15 cm x 4.6 mm) (Spherisorb S30DS2; Phase separations, Clwyd, UK), with a
255 mobile phase of MeOH:water (97:3 v/v) at a flow rate of 1.1mL min⁻¹. Fluorescence
256 detection (Dionex RF2000) was performed at 295nm (excitation) and 330nm (emission). The
257 α -tocopherol peak was identified and quantified by comparison with a standard solution of α -
258 tocopherol (T3251 Sigma-Altrich) in methanol. Total carotenoid and α -tocopherol
259 concentrations are reported as $\mu\text{g g}^{-1}$ yolk.

260 **Statistical analyses**

261 To test the influence of winter provisioning on egg production, general linear mixed models
262 (GLMM) were applied to the following response variables: lay date; clutch size; clutch and
263 egg mass; and yolk α -tocopherol and total carotenoid concentrations. A $\log_{10}:\log_{10}$ GLMM of
264 yolk mass on egg mass was used to examine proportionality of yolk investment. Nest box
265 identity nested within woodland site was specified as the random term, to control for temporal
266 and spatial pseudoreplication. An information-theoretic approach based on Akaike's
267 Information Criterion (AIC) was then used for model selection and model averaging
268 (Burnham & Anderson 2002), appropriate for complex large-scale field investigations as
269 reported here (Whittingham *et al.* 2006).

270

271 All first clutches (n= 467) were included in lay date analysis, whilst clutch and egg
272 component analyses excluded clutches with laying breaks >2 days (n= 23) and/or abandoned
273 before incubation (n= 32). Eggs showing any sign of incubation upon dissection (i.e. visible
274 early-stage embryo) were excluded from egg component analyses (16% of eggs collected).
275 For each analysis, a candidate set including all possible models given the predictor variables
276 (Table 1), plus a null model fitted with only the intercept, were compared. A quadratic
277 function of lay date (lay date squared) was initially included in to test for non-linear
278 relationships, but it did not improve model fit according to AICc and had little predictive
279 power (main effect parameter estimate (β) < 0.0001 in all cases) and was therefore excluded
280 from further analyses. Normality and homoscedasticity of residuals were checked prior to
281 model selection; concentrations of α -tocopherol and total carotenoids were subsequently log-
282 transformed to correct normality.

283

284 Models were compared using AICc (i.e. AIC corrected for small sample size), where the best
285 fitting model has the lowest AICc value and all other models are ranked according to their
286 difference in AICc from the top model (ΔAICc). If a single ‘best’ model could not be
287 identified, model averaging was applied across the most strongly supported models (the
288 confidence set), defined by $\Delta\text{AICc} \leq 2.0$ (Burnham & Anderson 2002). Akaike weights (w_i)
289 were used to assess the relative support of models within a confidence set, calculate model-
290 averaged parameter estimates (β) and associated standard errors (SE) and estimate relative
291 importance of explanatory variables (w). The predictive power of top ranking models was
292 assessed by calculating a pseudo- R^2 value following Nagelkerke (1991), since coefficient of
293 determination (R^2) cannot be generated directly for mixed models. Where treatment (or a
294 specific treatment interaction) was well supported for inclusion in the best model, further
295 testing was applied to assess between treatment group differences. Using AICc, the top
296 GLMM model within the confidence set was compared to replicate models in which two focal
297 treatment groups were paired. In this instance, strong support for a between treatment group
298 difference was concluded if the model in which the two groups were paired was $\Delta\text{AICc} > 2$
299 from the original GLMM model. All statistical analyses were conducted in R version 2.12.2
300 (R Development Core Team 2011) using libraries nlme (Pinheiro *et al.* 2010) and MuMIn
301 (Bartoń 2011).

302 **RESULTS**

303 **Timing of laying**

304 Lay dates did not differ between treatment groups, but were strongly predicted by between-
305 year differences (mean \pm SE per year: 26.6 \pm 0.6; 24.7 \pm 0.5; 30.2 \pm 0.5 respectively, where 1= 1
306 April). The top model, featuring year only ($w_i= 0.993$, $n= 467$, pseudo- $R^2= 0.109$), was at

307 least 141 times better supported by the data than all alternatives within the candidate set
308 which also included treatment and the treatment \times year interaction ($w_i > 0.007$).

309 **Clutch size and relative mass of egg components**

310 There was strong support for an effect of treatment on the relationship between egg mass and
311 yolk mass; all models within the confidence set for proportionality of yolk investment
312 included the treatment \times \log_{10} (egg mass) interaction (Table 2, $n = 299$, Fig. 1). *Post-hoc*
313 testing revealed that this was driven by differences between the fat and fat+VE treatment
314 groups ($\Delta AICc = 8.836$, for paired model against top model). Whilst fat-fed females had
315 proportionally small yolks compared to controls, fat+VE females produced proportionally
316 larger yolks as egg mass increased (Table 3). But there was no evidence that proportional
317 yolk investment differed between the control group and the fat ($\Delta AICc = 1.672$) or the fat+VE
318 treatment groups ($\Delta AICc = 0.413$). The importance of year, clutch size and lay date in the
319 model was relatively smaller (Table 3).

320

321 Winter provisioning treatment explained less than half the variation in clutch size compared
322 to year or lay date when excluding possible interactions ($w = 0.435$; Table 2, models 1 and 2
323 only, $n = 413$) and was poorly estimated as indicated by relatively high standard errors (Table
324 3).

325

326 Variation in total resource deposition was investigated in an analysis of clutch mass,
327 controlling for clutch size. Clutch mass was not influenced by treatment and similarly neither
328 was individual egg mass (Table 2, $n = 388$ and 312 respectively; no models contained
329 treatment or treatment-interactions within 2 $\Delta AICc$ confidence sets). Year was the best
330 predictor of total clutch and individual egg mass variation; females laid clutches of reduced

331 mass in 2009 and had comparatively large eggs in 2008 ($w= 1.000$; Table 3). However the
332 models within the egg mass confidence set explained only a small proportion of the variation
333 (pseudo- $R^2= 0.046$ and 0.047 ; Table 2). Furthermore, a variance components analysis of
334 model 1 (Table 2), using restricted maximum likelihood (REML), revealed that 71.7% of egg
335 mass variation was attributed to inter-clutch variation, with woodland site accounting for
336 3.5% and nest box for 24.9%, indicating that inherent differences among females were the
337 greatest predictor of egg mass variation.

338 **Egg yolk antioxidants**

339 Variation in yolk α -tocopherol concentration was not affected by winter provisioning
340 treatment. In this analysis the best supported model received a high level of support
341 compared to all others in the candidate set (unadjusted $w_i= 0.748$; Table 2), suggesting that
342 differences were the result of annual and seasonal variation. α -Tocopherol levels were lower
343 in 2009 and decreased as the season progressed, but to a greater extent in larger clutches
344 (Table 3).

345

346 By contrast, evidence of a difference in total carotenoid concentration between the treatment
347 groups received a high level of support in an interaction with lay date (Table 2). *Post-hoc*
348 comparisons revealed that yolk total carotenoid concentration in relation to laying date
349 differed between the fat-fed treatment group compared to fat+VE ($\Delta AICc= 12.346$, for paired
350 model against top model) and control groups ($\Delta AICc= 2.469$), whilst the fat+VE and control
351 groups were comparatively similar ($\Delta AICc= 1.580$) (Table 3, Fig. 2). Compared to females
352 of the other groups, fat-fed females invested fewer carotenoids into their eggs early in the
353 season, but more carotenoids later in the season. Both year and the lay date by clutch size
354 interaction also received strong selection probabilities, whereby total carotenoid levels were

355 reduced in 2010 and showed a seasonal increase, to a greater extent in smaller clutches ($w \geq$
356 0.540, Table 3).

357 **DISCUSSION**

358 The results of this study demonstrate pervasive, downstream effects of winter provisioning on
359 egg production the following spring. However, the effects were strongly influenced by the
360 types of nutrients provided. Previous studies of carry-over effects in birds have considered
361 that macronutrients such as fats are key limiting resources; an increase in dietary
362 macronutrient supply may either result in storage in body tissues for later use, or may result in
363 boosted body condition in one season or year, such that individuals perform better in a
364 subsequent season or year (reviewed by Harrison *et al.* 2011). Our results are striking because
365 they demonstrate that: 1) increased dietary fat availability in winter can in fact impair
366 subsequent egg production in terms of relative yolk mass, and egg carotenoid deposition early
367 in the breeding season; and 2) macronutrients such as fats are clearly not the only nutritional
368 currency that can invoke carry-over effects; negative effects of winter provisioning were not
369 seen in birds fed fat together with vitamin E.

370

371 It is well established that larger eggs confer benefits to offspring in terms of growth and
372 survival, with these effects attributed to yolk resources (Williams 1994). But furthermore, as
373 well as providing the major energetic requirements for early development, the yolk comprises
374 a cocktail of micronutrients and maternally-derived compounds known to influence offspring
375 fitness, such as antioxidants, immunoglobulins and hormones (Gasparini *et al.* 2001; Blount
376 *et al.* 2002b; Groothuis *et al.* 2005). Thus, increasing yolk mass can benefit offspring in
377 terms of enhanced embryonic growth and post-hatching reserves (Peach & Thomas 1986;
378 Bourgault *et al.* 2007). Egg size is a relatively inflexible trait within females, compared to

379 between-individual differences (Christians 2002). Consistent with this we found no effects of
380 winter provisioning treatment on egg mass *per se*. However, variation in the yolk component
381 as a function of total egg mass reflects the absolute difference in the nutrient and energy
382 content of an egg. As such relative yolk mass is an important determinant of egg quality
383 (Williams 1994), which females may modulate adaptively or due to constraint, depending on
384 their physiological condition and access to resources. Typically in altricial and precocial bird
385 species, yolk mass varies in direct proportion to egg mass (i.e. an isometric relationship;
386 Williams 1994). For a small proportion of females constrained to lay small eggs, fat
387 provisioning appears to have been beneficial. But, as egg mass increased fat-provisioning led
388 to a significant decline in relative yolk mass (negative allometry; Fig. 1). However this
389 deleterious consequence of winter provisioning was not seen in birds that had received fat
390 together with vitamin E.

391

392 How could winter provisioning with fat apparently reduce the capacity of females to produce
393 large egg yolks? As income breeders, blue tits are incapable of storing sufficient amounts of
394 endogenous macronutrients to fuel reproduction (Drent & Daan 1980), therefore fat
395 provisioned in winter is unlikely to have been utilised directly for egg formation several
396 weeks or months later. Instead, yolk mass is a function of food availability in the days
397 leading up to laying in income breeders (Ardia, Wasson & Winkler 2006). It therefore
398 appears that winter provisioning with fat subsequently impaired the capacity of birds to
399 acquire, assimilate and/or mobilise key resources required for yolk formation during egg
400 production. Yet, the addition of vitamin E to fat supplements mitigated this. We think the
401 most likely explanation is that birds may come to rely on readily accessible provisioned foods
402 in winter (Brittingham & Temple 1992). Whilst a high-fat diet provides an abundance of
403 energy, it could mean that birds fail to obtain a balanced, natural diet and are lacking in key

404 nutrients such as antioxidants. Indeed, a high fat diet should increase the requirement for
405 antioxidant protection, polyunsaturated fatty acids in particular being highly susceptible to
406 oxidative damage (Igosheva *et al.* 2010). In our study it was logistically impossible to follow
407 individual birds throughout winter and spring, and thus we were unable to assess effects of
408 provisioning on oxidative stress levels during winter and to relate this to breeding
409 performance. However, it seems possible that fat-fed females may have suffered oxidative
410 stress which impaired their ability to invest resources in egg composition. Vitamin E is a
411 potent antioxidant, capable of breaking the chain of lipid peroxidation (Surai 2007), and
412 therefore acquisition of dietary vitamin E could have mitigated the oxidative burden imposed
413 by a fatty diet.

414

415 Yolk α -tocopherol concentrations reduced over the laying period and were unaffected by
416 winter provisioning treatment. It is therefore unlikely that α -tocopherol acquired through
417 winter provisioning was stored for later use during egg production. We found no significant
418 effect of provisioning treatment on laying date. However, compared to both unfed and
419 fat+VE females, individuals provisioned with fat alone produced eggs with relatively low
420 concentrations of carotenoids early in the season, but relatively high concentrations of
421 carotenoids later in the season. There is strong selection for breeding early in blue tits, as in
422 many other bird species, because early breeders afford their offspring higher survival and
423 recruitment prospects (Perrins 1996). It has been shown that supplementing the diet of blue
424 tits with carotenoids just prior to and during laying results in significantly elevated levels of
425 carotenoids in eggs (Biard, Surai & Møller 2005). Increased levels of yolk carotenoids can
426 counter lipid peroxidation (Blount *et al.* 2002a; McGraw, Adkins-Regan & Parker 2005), to
427 which the lipid-rich yolk and rapidly growing embryo are highly susceptible, and have a
428 range of beneficial effects in nestlings such as increased immunity and survival (Saino *et al.*
429 2003; Biard, Surai & Møller 2005; McGraw, Adkins-Regan & Parker 2005). However,

430 females in poor condition or experiencing oxidative stress may deposit fewer carotenoids into
431 their eggs in favour of somatic maintenance (e.g. Hörak, Surai & Møller 2002; Blount *et al.*
432 2004; Isaksson, Johansson & Andersson 2008). It is perhaps more important to lay early than
433 to produce eggs that contain high levels of carotenoids. It seems likely that in our study, fat-
434 fed birds attempted to lay at the optimal time even though it was at the cost of producing eggs
435 that contained relatively low levels of carotenoids. Potentially, this could be amplified if
436 winter provisioning enabled relatively low-quality individuals to enter the breeding
437 population, which would otherwise not have bred at all. In the fat-fed treatment group, where
438 nutrient acquisition appeared to have deleterious consequences for egg production, a change
439 in phenotypic structuring could have resulted in reduced average egg quality at the population
440 level.

441

442 It is possible that the influence of winter provisioning reduced as the season progressed, due
443 to the increase in time between food uptake and egg laying. However, we do not know why
444 fat-fed birds that laid late produced eggs with relatively high concentrations of carotenoids,
445 compared to females of the other provisioning treatments. It is well established that dietary
446 access to carotenoids increases over the laying season in parids. Caterpillar supply, which
447 provides the main food resource for breeding tits, increases in number and quality across the
448 laying period (Arnold *et al.* 2010). The increase in carotenoid allocation to eggs later in the
449 season in fat-fed birds was not simply because they produced smaller clutches; although
450 clutch size decreased over the laying season, this decline did not differ significantly amongst
451 provisioning treatments. One possible explanation is that fat-fed birds had low survival
452 prospects, i.e. no expectation of future reproduction, and therefore invested more in current
453 reproduction as the season neared its end (Royle, Surai & Hartley 2003; Alonso-Alvarez *et al.*

454 2004). We do not have the data to assess this possibility, but this would be an interesting
455 direction for further work.

456

457 As urban land cover expands, gardens are expected to play an increasingly important role in
458 the conservation of biodiversity (Chamberlain, Cannon & Toms 2004). The provision of food
459 for garden birds has been thought likely to benefit this objective (e.g. Toms & Sterry 2008),
460 although there is limited and indeterminate evidence of its ecological impacts (e.g. Robb *et al.*
461 2008b; Harrison *et al.* 2010). More generally, food provisioning has also been applied as a
462 conservation tool to manage endangered populations (e.g. Armstrong, Castro & Griffiths
463 2007; Oro *et al.* 2008). Our study is the first to report deleterious effects of provisioning
464 which were carried over from one season to the next. We emphasise, however, that our study
465 focussed on egg phenotypes; it will be important to see how these effects translate into fitness
466 consequences. The mechanism by which these negative effects were generated is of key
467 importance; the provision of energy-rich fat supplements in winter had negative
468 consequences for female egg investment several weeks after provisioning stopped. Yet
469 at the population level this was mitigated by the provision of fat together with vitamin E.
470 This is the first direct evidence that the specific nutritional composition of provisioned foods
471 may determine whether carry-over effects on breeding performance are positive or negative at
472 the population level. Therefore, where provisioning is practiced as a conservation tool, careful
473 consideration should be given to the nutritional composition of foods. Whether winter
474 provisioning of garden bird species is considered to be beneficial or deleterious may depend
475 on whether effects are interpreted at the level of individuals, or populations. Provisioning
476 may lead to a reduction in average levels of egg quality at the population level. However, if
477 provisioning enables certain low-quality individuals to breed, when they might otherwise
478 have died or survived only as non-breeders, this would clearly enhance their lifetime

479 reproductive success and may in fact boost the overall population size. It is evident that
480 further work at the level of individuals is needed to understand how winter feeding may be
481 used to benefit wild bird populations in the future.

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660

661 **Table 1.** List of parameters included as fixed explanatory variables in GLMMs to investigate
662 causes of egg production variation. Egg production response variables examined were lay
663 date (LD); clutch size (CS); clutch mass (CM); egg mass (EM); and yolk α -tocopherol
664 (YTOC) and total carotenoid (YCAR) concentrations

Fixed predictors	Description/levels	Reason for inclusion	Response
Treatment	3 level factor – unfed, fat, fat+VE	Examine the effect of winter provisioning	LD; CS; CM; EM; YTOC; YCAR; YM
Year	3 level factor – 2008, 2009, 2010	Life-history traits vary annually (Svensson & Nilsson 1995; Lambrechts <i>et al.</i> 2004)	LD; CS; CM; EM; YTOC; YCAR; YM
Lay date	Continuous, 1 = 1 April	Clutch size and breeding performance vary seasonally (Norris 1993; Perrins 1996)	CS; CM; EM; YTOC; YCAR; YM
Clutch size	Continuous	Account for variation in female breeding condition and trade-offs between egg number and quality (Slagsvold & Lifjeld 1990; Perrins 1996)	CM; EM; YTOC; YCAR;
Log ₁₀ (Egg mass (g))	Continuous	Examine proportionality of yolk investment	YM
Treatment × Year	2-way interaction	Examine whether effects of annual variation were consistent between treatments	LD; CS; CM; EM; YTOC; YCAR;
Treatment × Lay date	2-way interaction	Examine whether effects of seasonal variation were consistent between treatments	CS; CM; EM; YTOC; YCAR;
Treatment × Clutch size	2-way interaction	Examine whether effects of clutch size were consistent between treatments	CM; EM; YTOC; YCAR;
Lay date × Clutch size	2-way interaction	Improve model fit by controlling for decline in clutch size through laying period (Perrins 1996)	CM; EM; YTOC; YCAR;
Treatment × Log ₁₀ (Egg mass)	2-way interaction	Examine whether proportional yolk investment was consistent between treatments	YM

665

666 **Table 2.** Confidence sets of ranked models for analyses of maternal investment in egg
667 production, based on Akaike's information criterion corrected for small sample size (AICc)

Rank	Model parameters*	Log-likelihood	AICc	Δ AICc	w_i †	Pseudo- R^2
<i>(a) Proportional yolk mass ($\log_{10}(\text{yolk mass})$)</i>						
1	(treat \times $\log_{10}(\text{EM})$)	581.7	-1144.8	0.000	0.228	0.486
2	(treat \times $\log_{10}(\text{EM})$) + year	583.8	-1144.7	0.027	0.225	0.494
3	(treat \times $\log_{10}(\text{EM})$) + LD	582.4	-1144.1	0.636	0.166	0.489
4	(treat \times $\log_{10}(\text{EM})$) + CS	584.3	-1143.8	0.993	0.139	0.489
5	(treat \times $\log_{10}(\text{EM})$) + year + CS	584.3	-1143.6	1.215	0.124	0.496
6	(treat \times $\log_{10}(\text{EM})$) + year + LD	584.3	-1143.4	1.344	0.117	0.496
<i>(b) Clutch size</i>						
1	year + LD	-756.8	1527.9	0.000	0.444	0.142
2	year + LD + treat	-755.0	1528.4	0.530	0.340	0.149
3	year + (treat \times LD)	-753.3	1529.3	1.443	0.216	0.156
<i>(c) Clutch mass</i>						
1	year + LD + CS	-439.14	894.7	0.000	0.717	0.857
2	year + (LD \times CS)	-439.02	896.5	1.857	0.283	0.857
<i>(d) Egg mass</i>						
1	year + CS	294.3	-574.2	0.000	0.705	0.046
2	year + CS + LD	294.5	-572.5	1.746	0.295	0.047
<i>(e) α-Tocopherol concentration</i>						
1	year + (LD \times CS)	-210.61	439.8	0.000	1.000	0.111
<i>(f) Total carotenoid concentration</i>						
1	year + (LD \times CS) + (treat \times LD)	-79.00	185.2	0.000	1.000	0.252

668 * Treat, winter feeding treatment; EM, egg mass; LD, lay date; CS, clutch size; \times , interaction term

669 † Akaike weight for the model within the confidence set

670 **Table 3.** Relative variable importance (w), model-averaged parameter estimates (Est.) and standard errors (SE) for variables represented in the
671 confidence sets of maternal egg investment analyses

Parameter	Proportion yolk			Clutch size			Clutch mass			Egg mass			α -Tocopherol concentration			Total carotenoid concentration		
	w	Est.	SE	w	Est.	SE	w	Est.	SE	w	Est.	SE	w	Est.	SE	w	Est.	SE
Intercept		-0.678	0.014		10.931	0.487		1.407	0.491		1.253	0.037		4.189	0.508		2.635	0.364
Clutch size	0.263	0.000	0.001				1.000	1.035	0.054	1.000	-0.008	0.003	1.000	0.125	0.057	1.000	0.110	0.039
Lay date	0.283	0.000	0.000	1.000	-0.096	0.015	1.000	-0.020	0.015	0.295	0.000	0.001	1.000	0.035	0.017	1.000	0.037	0.012
Log ₁₀ (egg mass)	1.000	0.870	0.102															
Treatment	1.000			0.556												1.000		
fat		0.007	0.010		-0.369	0.681											-0.315	0.176
fat+VE		-0.009	0.010		-0.255	0.424											0.203	0.167
Year	0.466			1.000			1.000			1.000			1.000			1.000		
2009		0.004	0.006		-0.332	0.182		-0.289	0.094		-0.033	0.013		-0.134	0.066		-0.002	0.042
2010		0.004	0.006		0.305	0.183		-0.069	0.094		-0.027	0.013		0.118	0.067		-0.159	0.043
Lay date \times clutch size							0.283	0.000	0.002				1.000	-0.006	0.002	1.000	-0.004	0.001
Treatment \times lay date				0.216												1.000		
fat \times lay date					0.010	0.022											0.014	0.006
fat+VE \times lay date					0.002	0.012											-0.009	0.006
Treatment \times log ₁₀ (egg mass)	1.000																	
fat \times log ₁₀ (egg mass)		-0.188	0.138															
fat+VE \times log ₁₀ (egg mass)		0.225	0.140															

672 **LEGEND TO FIGURES**

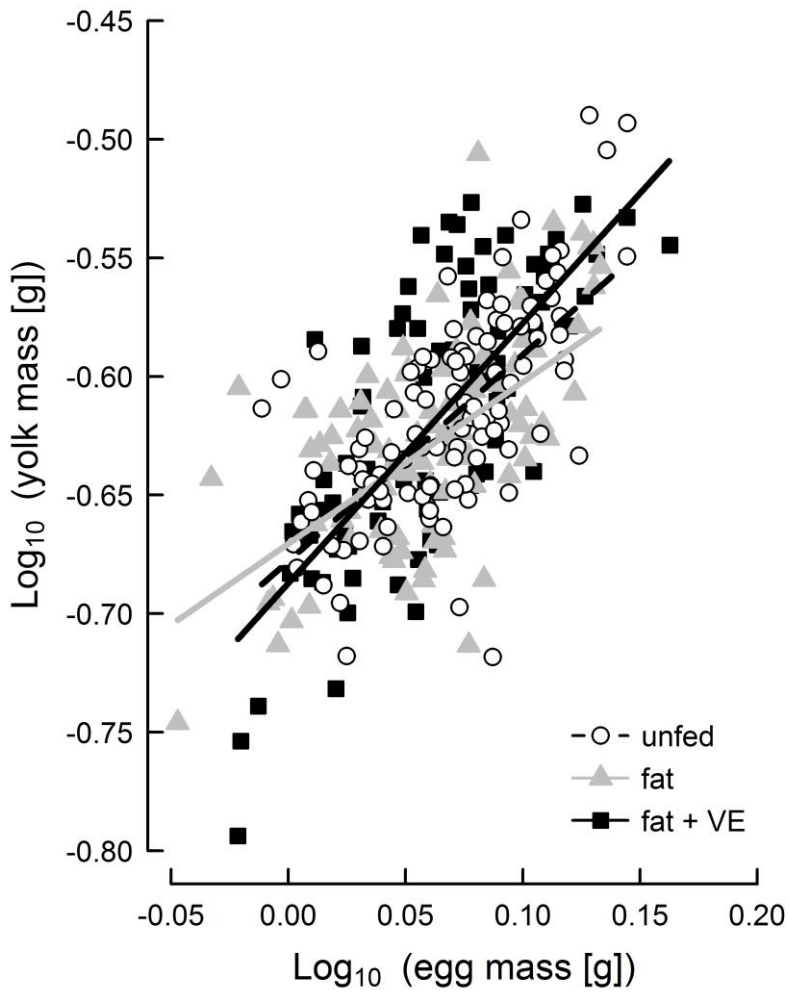
673 **Figure 1.** Relationship between yolk mass and egg mass. Lines fitted using model averaged
674 parameter estimates. Testing the difference in the slopes (b) against 1.0 (isometry) reveals
675 that increases in egg mass are accompanied by a proportionate increase in yolk mass for
676 control ($b= 0.870 \pm 0.102(\text{SE}); t_{103}= 1.23, p= 0.200$) and fat+VE ($b= 1.095 \pm 0.138; t_{92}= 0.69,$
677 $p= 0.492$) groups. But yolk mass increases proportionately less than egg mass in the fat group
678 (negative allometry; $b= 0.682 \pm 0.135; t_{98}= 2.35, p= 0.021$).

679

680 **Figure 2.** Relationship between yolk total carotenoid concentration and lay date. Lines fitted
681 using model averaged parameter estimates. See Tables 1 and 2 for statistical findings and text
682 for details.

683

684 **Figure 1.**



685

686 **Figure 2.**

