



Do Barn Swallow nestlings incur an oxidative cost of begging?

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Abstract

Some theoretical models predict that nestling begging must be a costly activity to transmit reliable information to parents about offspring hunger. One candidate cost is oxidative stress, which could impair growth or immune function. This hypothesis predicts that nestlings in a poor oxidative status should pay higher costs for a given amount of begging, hence beg at lower rates for a similar degree of hunger. To test this prediction, we experimentally induced a transient alteration of the oxidative status of Barn Swallow (*Hirundo rustica*) nestlings by administering them either a single dose of a pro-oxidant substance (BSO) or an antioxidant (vitamin E), and recorded their begging behaviour. Chicks treated with vitamin E increased their begging rates in the hours following treatment. However, and contrary to prediction, BSO nestlings did not decrease begging compared to controls. These inconsistent results are difficult to explain by the hypothesis that begging performance is mediated by the oxidative status of nestlings because chicks with an impaired oxidative status (the BSO group) begged the same as controls. Alternatively, vitamin E may alleviate the inhibitory effects on begging of stress responses mediated by glucocorticoids. This study suggests that oxidative costs of begging are minor or absent in this species. Future studies should consider alternative explanations for an association between oxidative status, antioxidant supplementation and begging behaviour, particularly those involving glucocorticoids.

Keywords Begging · Honest signalling · Oxidative stress · *Hirundo rustica* · Vitamin E · BSO · Glucocorticoids

Zusammenfassung

Entstehen den Nestlingen von Rauchschnalben durch Betteln oxidative Kosten?

Es gibt theoretische Modelle, die besagen, dass das Betteln von Nestlingen vermutlich eine kostenintensive Art ist, um den Eltern zuverlässige Information über den Hunger der Jungen zu geben. Ein möglicher Kostenfaktor wäre oxidativer Stress, der das Wachstum oder das Immunsystem beeinträchtigen könnte. Diese Hypothese besagt, dass Nestlingen in einem schlechten oxidativen Zustand höhere Kosten für ein bestimmtes Maß an Betteln entstehen und sie deshalb bei gleichem Hunger besser weniger häufig betteln sollten. Um dies zu testen, führten wir experimentell eine vorübergehende Veränderung des Oxidationsstatus von Nestlingen der Rauchschnalbe (*Hirundo rustica*) herbei, indem wir ihnen entweder eine einmalige Dosis einer pro-oxidativen Substanz (BSO) oder eines Antioxidans (Vitamin E) verabreichten und ihr Bettelverhalten aufzeichneten. Die mit Vitamin E behandelten Küken bettelten in den Stunden nach der Behandlung häufiger. Aber entgegen der Vorhersage bettelten die BSO-Küken im Vergleich zu den Kontrollen nicht weniger. Diese widersprüchlichen Ergebnisse

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lassen sich nur schwer mit der Hypothese erklären, dass die Bettelaktivität durch den oxidativen Status der Nestlinge gesteuert wird, da Küken mit einem schlechteren oxidativen Status (die BSO-Gruppe) genauso viel bettelten wie die Kontrollgruppe. Andererseits wäre es möglich, dass Vitamin E die durch Glukokortikoide hervorgerufene hemmende Wirkung auf die Entstehung von Stressreaktionen verringert. Unsere Studie deutet darauf hin, dass dieser Spezies durch das Betteln kaum oder gar keine oxidativen Kosten entstehen. Künftige Untersuchungen sollten alternative Erklärungen für einen Zusammenhang zwischen oxidativem Status, der Zufuhr von Antioxidantien und dem Bettelverhalten in Betracht ziehen, insbesondere solche, die Glukokortikoide berücksichtigen.

Introduction

In birds with parental care, nestlings usually display to solicit food from their parents with conspicuous behaviours including body postures and vocalizations (Kilner and Johnstone 1997; Budden and Wright 2001). Theoretical work suggests that begging signals have evolved as a costly mechanism of resolution of parent–offspring conflict over the amount and distribution of food resources (Godfray 1991; Parker et al. 2002). In particular, theoretical models that postulate that begging displays evolved as information-rich honest signals assume that begging entails a cost that reduces nestling fitness in direct proportion to the degree of signal expression in order to maintain honesty (Brilot and Johnstone 2003). However, other theoretical possibilities assuming cheaper, less informative begging signals are also feasible (Lachmann et al. 2001; Számadó 2011).

Despite considerable research effort over the last four decades, empirical evidence for begging costs remains elusive, at least for birds (Moreno-Rueda 2007; Stynoski et al. 2018). While spontaneous begging seems to incur little costs in terms of energetic expenditure (Chappell and Bachman 2002), some laboratory studies found that experimentally enforced begging can reduce nestling growth and immune function (Redondo et al. 2016). There is compelling evidence that oxidative status plays a central role in biological processes (Costantini 2019). Reactive Oxygen Species (ROS) are potentially toxic by-products of cellular catabolism that can impair different physiological processes (Halliwell and Gutteridge 2007). To prevent oxidative stress, organisms must compensate the oxidative actions of ROS with antioxidant defences. The balance between oxidants and antioxidants (i.e., the oxidative status) can mediate life-history trade-offs and affect the fitness of an individual (Costantini 2008; Monaghan et al. 2009; Metcalfe and Alonso-Alvarez 2010; Garratt and Brooks 2012). It has been hypothesized that growth and immune begging costs found in laboratory studies are mediated by oxidative stress under the hypothesis that protracted begging (like growth or immune responses; Hasselquist and Nilsson 2012) is a source of oxidative imbalance (Noguera et al. 2010; Moreno-Rueda et al. 2012; Royle et al. 2015). In magpie (*Pica pica*) nestlings under laboratory conditions, enforced

begging rates and oxidative damage correlated positively (after controlling for mass gained and immune response), suggesting that begging activity may directly contribute to the production of ROS or increase the demand of ROS scavengers (Moreno-Rueda et al. 2012).

Supporting evidence for an oxidative cost of begging remains equivocal at present. Noguera et al. (2010) found that yellow-legged gulls (*Larus michahellis*) chicks experimentally supplemented with an antioxidant (vitamin E) displayed higher rates of chatter calls (but not other begging components like pecking or pee calls), mainly in those chicks with a lower body size. However, in red-winged blackbirds (*Agelaius phoeniceus*), great tits (*Parus major*) and pied flycatchers (*Ficedula hypoleuca*), antioxidant supplementation did not affect nestling begging rates (Hall et al. 2010; Maronde and Richner 2015; Parejo-Pulido et al. 2023). A second study on great tits found that female nestlings begged significantly less than males in control nests, whereas both sexes begged at similar rates in vitamin E-supplemented nests (Maronde et al. 2018). Other studies had also found mixed results between the antioxidant capacity of yolk and the begging behaviour of nestlings shortly after hatching (Rubolini et al. 2006; Helfenstein et al. 2007 see also Tschirren et al. 2005). In Barn Swallows, a correlational study found that begging bout duration and postural intensity at 14–15 days decreased with oxidative damage measured at 12 days, but in a context-dependent manner (only after food deprivation and when nest mates were genetically related, respectively; Boncoraglio et al. 2012). However, positive effects of vitamin E supplementation on nestling growth in this species did not depend on brood size manipulation (de Ayala et al. 2006) despite Barn Swallow (*Hirundo rustica*) nestlings beg more in larger broods (Lotem 1998; Saino et al. 2000), which contradicts the hypothesis that availability of vitamin E and other antioxidants depends on begging effort.

In this study, we test whether oxidative stress is a mechanism underlying begging behaviour (Noguera et al. 2010) in Barn Swallows. To this aim, we experimentally induced a transient alteration of the oxidative status of nestlings under otherwise natural conditions and recorded their begging output during the following hours. Begging displays are dynamic signals that convey information about variations in nestling hunger and nutritional condition over short

timescales (Gurguis and Duckworth 2022). Hence, if begging performance correlates with the oxidative status of nestlings, and this association is relevant for maintaining signal reliability, we expect this co-variation to occur on a short temporal scale. We used a stratified block design whereby nestlings within a brood were assigned to three different experimental treatments. First, some nestlings were injected with a specific inhibitor (DL-buthionine-S,R-sulfoximine BSO) of the biosynthesis of glutathione, the most important intracellular antioxidant (Griffith 1982) in order to temporarily impair their oxidative status. Glutathione depletion further handicaps the action of other antioxidants, particularly ascorbic acid, which rely on glutathione reactions for proper function (Koch and Hill 2017). This is the first time that the hypothesis is tested by inducing an impairment, rather than an enhancement, of the oxidative status of nestlings. Second, other nestmates were supplemented with vitamin E, an antioxidant that protects against lipid peroxidation and improves antioxidant machinery in bird chicks (Tsai et al. 2008; Sodhi et al. 2008). Finally, the remaining nestmates were treated as controls. If begging solicitation is constrained by oxidative stress (Boncoraglio et al. 2012) or limited by the availability of antioxidants (Noguera et al. 2010), we predicted that BSO nestlings would beg at lower rates than controls and vitamin E nestlings. Depending on whether natural antioxidants and antioxidant defences of chicks were limited or not, vitamin E nestlings should beg more or the same as controls, respectively.

Material and methods

Experimental procedure

The study was conducted in a Barn Swallow population breeding at the Córdoba University Campus. Nests were regularly inspected from middle April to early June to determine hatching dates and brood size. To monitor nestling behaviour, we placed a small mirror above the nest 3–4 days prior to the experiment to minimize disturbances on the day of recording. The experiment was performed with 81 chicks from 21 nests, when nestlings were 5–9 days old (day 1 = hatching of the oldest nestling). Nestlings attain maximum growth rates at days 7–8 (Fernaz et al. 2012) and vitamin E supplementation at this age (but not others) significantly improves nestling mass and condition (de Ayala et al. 2006). Brood size ranged from 2 to 5 chicks. Nestling mass was measured with an electronic balance to the nearest 0.1 g. Chicks were individually marked with a numeric code on the head using non-toxic waterproof markers.

Within each brood, nestlings were ranked according to their mass and assigned to either a BSO injection, vitamin E supplementation, or control treatment. Begging behaviour

and parental food allocation in Barn Swallows are affected by several factors including nestling age (Bonisoli-Alquati et al. 2011), sex (Saino et al. 2003), nestling condition or quality (De Ayala et al. 2007; Romano et al. 2011, 2016) and relative hunger level compared to nestmates (Romano et al. 2012). Many such factors (age, quality and nutritional condition) are closely related to birth order, which can be inferred from nestling mass ranks. We changed the order of treatments according to mass rank between successive broods in order to ensure that our sample was unbiased with respect to mass rank and sex, as well as balanced across different brood sizes (i.e., the heaviest nestling in the first brood was given BSO and the second acted as control, etc.; for the second brood, the heaviest nestling acted as control and the second was given vitamin E, etc.). Treatments were administered in late morning (11:00–12:00 h). BSO-chicks (N = 26) received a subcutaneous injection into the back of 0.1 ml of a 70 mg/ml water solution of BSO (DL-buthionine-S,R-sulfoximine; Sigma, ref. B2640) and one drop of peanut oil (Sigma, ref. P2144) orally. BSO dosage and administration protocol followed previous work with great tit nestlings (Galván and Alonso-Alvarez 2008) and is equivalent to a 3.1 $\mu\text{mol/g}$ dose for a 10-g nestling. This dosage was chosen because previous studies with wild bird chicks that found significant effects of BSO on glutathione depletion did it with lower (1–2 $\mu\text{mol/g}$ in Marchionatti et al. 2001) or similar dosages (41–81 mg/ml for great tit nestlings of a similar size in Galván & Alonso-Alvarez 2008; 2.25–4.50 $\mu\text{mol/g}$ for an average 8-g nestling). Vitamin E-chicks (N = 25) received an injection of distilled water and one drop of peanut oil enriched with vitamin E orally (dl- α -tocopherol acetate; Chiesi España, Barcelona, Spain) (112 mg/kg of body mass, Noguera et al. 2010). Finally, control chicks (N = 26) received an injection of distilled water and one drop of peanut oil.

Video analysis

Nestling behaviour was recorded with a high-definition video camera (Canon HF S21) during the afternoon, starting four hours after chicks had received the experimental treatment. This time frame was chosen to coincide with the period when glutathione depletion after BSO administration in mice is maximal (2–5 h) and well before recovering to baseline levels (Griffith 1982). Recording sessions had a mean duration of 165.5 min (± 7.9 SE), which is longer than duration of recordings in previous studies (90 min) (Boncoraglio et al. 2008; Bonisoli-Alquati et al. 2011; Romano et al. 2011, 2012, 2015, 2016). Our recording system focused on nestling heads to allow clear identification of each nestling in a brood during begging bouts. However, it often omitted details of nestling tarsi and wings, so we could not unambiguously measure begging postural intensity as in previous studies (Romano

et al. 2012, 2015, 2016). We counted the number of postural begging displays by each nestling and the number of feedings it received. A begging display was defined as a nestling gaping and stretching in the direction of incoming parents. Those events when nestlings simply opened their mouths (such as yawning) were not included. Begging displays performed when adults were both present and absent were recorded (Romano et al. 2015). A feeding was defined as an adult placing food into a nestling mouth and not removing it afterwards.

Statistical analyses

Statistical analyses were conducted in R 4.0.2 (R Core Team, 2020). All independent variables were Z-transformed (mean centered with SD of 1) to improve the stability of models, likelihood of model convergence, and the accuracy of parameter estimates (Harrison et al. 2018). Differences in nestling mass between experimental treatments were analyzed by linear mixed models with a Gaussian error distribution. Differences in begging rates and parental feeding counts were analyzed by generalized linear mixed models with a Poisson error distribution, including the actual observation time (in logarithms) as an offset term. To check model assumptions (e.g., overdispersion) and perform residual diagnostics for generalized models we used the 'DHARMA' package (Hartig 2017).

As candidate random effects we included nest (as an intercept), treatment (as a slope), and the correlation between them. To determine the optimal structure of random effects in models, we started with the most complex random structure (Barr et al. 2013) (including all fixed effects and their interactions in Gaussian models or none of them in generalized (non-gaussian) models). Then, we progressively removed random effects one by one, comparing these maximum likelihood models with the anova() function from the 'stats' package (Zuur et al. 2009). Nestling mass, test date (1 = recording date of first nest in the season), test time (daily hour) as well as their interactions with experimental treatment, were included as candidate fixed effects. The total number of parental visits (with or without feeding) was included as a fixed effect in the model predicting nestling begging rates. The number of begging displays performed by nestlings was included as a fixed effect in the model predicting parental feeding rates. To determine the best structure of fixed effects we removed effects one by one from the saturated model with the optimal random structure, starting from the least significant one. Then, we tested whether the removed effect contributed to explain variance in the response variable by maximum likelihood ratio test with the anova() function

as above (Zuur et al. 2009). Degrees of freedom for *t*-tests were estimated by Satterthwaite's method using the lmerTest package (Kuznetsova et al. 2017).

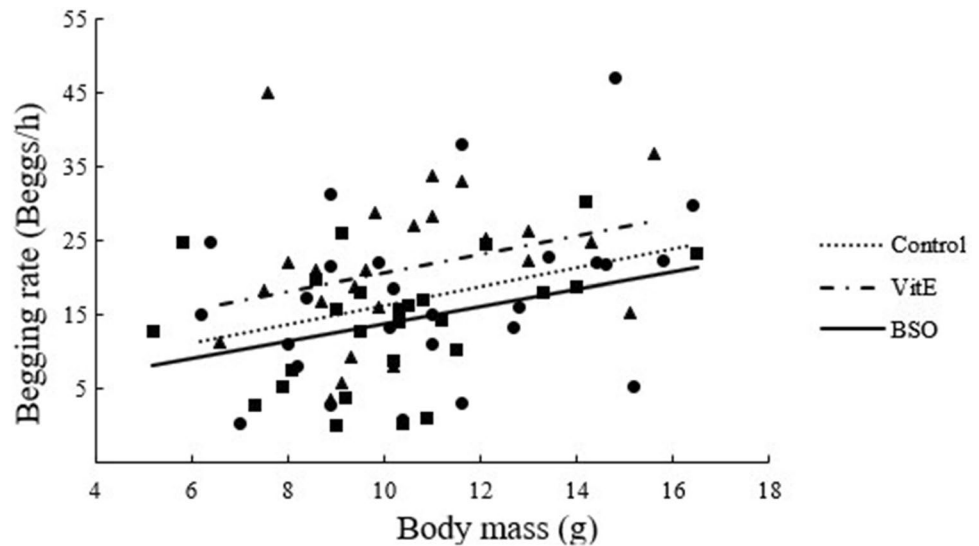
Results

Initial body mass of chicks in the BSO ($10.17 \text{ g} \pm 0.49 \text{ SE}$) and vitamin E ($10.43 \text{ g} \pm 0.47 \text{ SE}$) treatment groups did not differ from controls ($11.03 \text{ g} \pm 0.58 \text{ SE}$; linear mixed model with nest as random intercept, $t_{54,7} = -0.9$, $P = 0.372$ and $t_{54,7} = -0.95$, $P = 0.344$ respectively).

The optimal model to explain variation in the number of begging displays performed by chicks included as random effects nest (intercept) and treatment (slope). As fixed effects it included experimental treatment, number of parental visits and nestling mass. Nestlings begged more at nests more frequently visited by parents ($\beta = 0.45 \pm 0.090 \text{ SE}$, $Z = 5.05$, $P < 0.001$) and heavier chicks begged at lower rates ($\beta = -0.11 \pm 0.050 \text{ SE}$, $Z = -2.12$, $P = 0.034$). BSO chicks begged at similar rates than controls ($\beta = -0.14 \pm 0.117 \text{ SE}$, $Z = -1.17$, $P = 0.242$), but vitamin E chicks begged at marginally significant higher rates ($\beta = 0.25 \pm 0.133 \text{ SE}$, $Z = 0.90$, $P = 0.057$). Parental visits included both feeding and non-feeding visits. Begging rates may depend not only on parental stimulation but also on the actual feeding rate experienced by a nest. When we substituted number of total adult visits by number of individual feeding visits in the model, we found that nestlings fed more often begged more ($\beta = 0.07 \pm 0.013 \text{ SE}$, $Z = 5.39$, $P < 0.001$) and heavier chicks begged at lower rates ($\beta = -0.24 \pm 0.062 \text{ SE}$, $Z = -3.86$, $P < 0.001$). Vitamin E chicks begged at significantly higher rates ($\beta = 0.31 \pm 0.140 \text{ SE}$, $Z = 2.22$, $P = 0.026$) but BSO chicks begged at similar rates compared with controls ($\beta = -0.04 \pm 0.134 \text{ SE}$, $Z = -0.31$, $P = 0.753$). Since we used a within-brood experimental approach, variation in the number of parental visits affected all nestlings in a brood in a similar way irrespective of treatment (i.e. all treatments within a brood received the same number of visits). Therefore, we built a second model excluding the number of parental visits as a predictor. Again, heavier chicks tended to beg at marginally significant lower rates ($\beta = -0.09 \pm 0.052 \text{ SE}$, $Z = -1.71$, $P = 0.087$). BSO chicks begged at similar rates than controls ($\beta = 0.12 \pm 0.118 \text{ SE}$, $Z = -1.01$, $P = 0.311$), but vitamin E chicks begged at significantly higher rates ($\beta = 0.27 \pm 0.135 \text{ SE}$, $Z = 2.03$, $P = 0.0427$). Vitamin E nestlings displayed at an average rate of 21.30 begs/h (20.47–22.12 95% CI) while rates for control nestlings were 17.14 begs/h (16.05–18.23 95% CI) (Fig. 1).

The optimal model to explain variation in the number of parental feedings included nest as a random intercept

Fig. 1 Nestling begging rates according to nestling mass and experimental treatment: BSO (■); Vitamin E (▲); Controls (●)

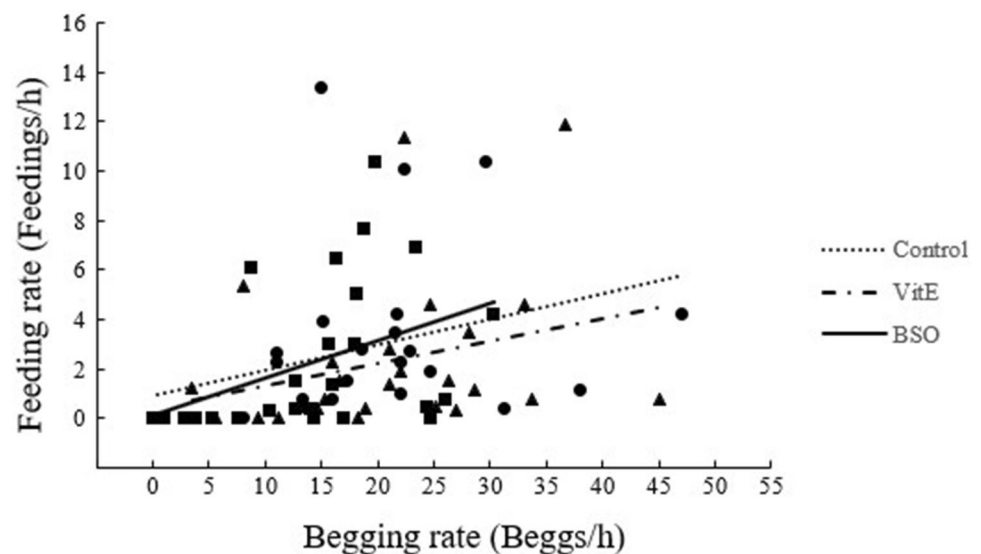


effect. As fixed effects, it included daily hour and nestling begging rate interacting with experimental treatment. Parents fed at lower rates later in the afternoon ($\beta = 0.61 \pm 0.234$ SE, $Z = -2.59$, $P = 0.009$). Parents fed more those nestlings begging at higher rates (marginal effect $\beta = 0.45 \pm 0.124$ SE, $Z = 3.64$, $P < 0.001$) but the relationship between feeding and begging was steeper for BSO chicks, relative to controls (interaction $\beta = 0.61 \pm 0.184$ SE, $Z = 3.35$, $P < 0.001$), than for vitamin E chicks ($\beta = -0.01 \pm 0.129$ SE, $Z = -0.01$, $P = 0.949$; Fig. 2). The relationship was similar for controls and chicks supplemented with vitamin E. Predicted feeding rates according to this model (all treatments pooled) for vitamin E supplemented and control chicks were 2.33 and 2.10 feedings/h, respectively.

Discussion

Our experimental treatment explained a significant (albeit small) fraction of inter-individual variance in begging performance by nestlings, even if the metric considered (all-or-nothing begging rates) was rather crude (e.g. compared with postural intensity, Kilner 2002) and included begging activity both in the presence and absence of adults (Romano et al. 2015). Chicks treated with vitamin E increased their begging rates but, contrary to our main prediction, BSO-treated chicks did not decrease begging rates compared to control nestlings. The effect size of vitamin E treatment ($\beta = 0.27$, 0.01–0.53 95% CI) was close to medium (0.30–0.49, Cohen 1988). Across all treatments, nestling begging rates explained parental feeding rates. The relationship between feeding rate and begging rate was similar for control and

Fig. 2 Adult feeding rates according to nestling begging rates and experimental treatment: BSO (■); Vitamin E (▲); Controls (●)



vitamin E-supplemented chicks, suggesting that, for a given displaying rate, begging displays of both chicks were equally efficient.

This result provides seemingly contradictory support for the hypothesis that oxidative status affects nestling begging behaviour. Though the oxidative status of nestlings following experimental treatment was not quantified, it is unlikely that nestling glutathione levels were unaltered during the observation period, given significant effects found for similar and lower dosages in other studies with bird chicks (Marchionatti et al. 2001; Galván and Alonso-Alvarez 2008; see Methods for details). Even a slight decrease in glutathione can induce a measurable shift in oxidative status (Koch and Hill 2017). A second possibility is that, even if glutathione levels were depleted, its timing did not coincide with begging trials (4 h after BSO injection). However, this time window was specifically chosen having in mind evidence about BSO pharmacodynamics. In mice, glutathione depletion in liver and kidney tissue following BSO administration is maximal at 2–5 h after injection and begins to recover 10 h later (Griffith 1982). Alternatively, our BSO treatment may have not altered the oxidative status of nestlings if they reallocated other endogenous antioxidant resources to compensate for glutathione depletion (Galván and Alonso-Alvarez 2008; Koch and Hill 2017). Note however that, in this case, nestlings could equally compensate for any transient unbalance in oxidative status caused by a burst of exaggerated begging activity, rendering begging signals unreliable to parents.

A final possibility is that the alteration of oxidative status following BSO injection had little or no effect on begging activity because oxidative stress is not a mechanism underlying begging behaviour. At first sight, this explanation seems at odds with studies reporting a positive effect of vitamin E on begging rates (Noguera et al. 2010; female nestlings in Maronde et al. 2018; this study). However, unlike BSO, which specifically targets a key component of the antioxidant machinery (Romero-Haro and Alonso-Alvarez 2020), vitamin E is involved in many other physiological processes from inflammation and cellular signalling to gene expression (Brigelius-Flohe 2009) and may affect nestling quality (e.g. growth or telomere dynamics) independently of its role as an antioxidant (Pérez-Rodríguez et al. 2019). Therefore, positive effects of vitamin E on begging may not necessarily qualify as supporting evidence for an oxidative begging cost if such effects are mediated by alternative physiological processes (e.g. growth) other than oxidative status. Two such processes may be relevant when interpreting mixed results found by different studies.

1. Growth. Chicks were younger in this study (ca. 50% of nestling period) and Noguera et al. (2010) (two days after hatching), compared with Maronde et al. (2018) (ca. 65% of nestling period), Hall et al. (2010) (ca. 70%), and

Maronde and Richner (2015) (ca. 76%) but similar to Parejo-Pulido et al. (2023). Younger nestlings may be more prone to oxidative stress (Costantini et al. 2006) but also grow at higher rates. In both this study Noguera et al. (2010) and Parejo-Pulido et al. (2023), chicks received intermediate doses of vitamin E (112 µg/g) compared to Hall et al. (2010) (600 µg/g for an estimated 35 g nestling mass) and the two studies in great tits (44–49 µg/g; Maronde and Richner 2015; Maronde et al. 2018). In Barn Swallows, supplementation with vitamin E at intermediate physiological doses improved nestling mass, body condition and feather growth, whereas higher doses did not enhance offspring quality compared to controls (de Ayala et al. 2006). However, only Hall et al. (2010) and Parejo-Pulido et al. (2023) reported significant effects of vitamin E treatment on nestling growth.

2. Stress response. Both in this study and Noguera et al. (2010), chicks experienced considerable manipulation by experimenters shortly before observations began. In the gull study, for example, chicks were captured on their nests and transported in textile bags to a hide outside the colony before recording their begging behaviour (Noguera et al. 2010). By contrast, in the remaining studies, nestlings were only marked (Hall et al. 2010; Maronde et al. 2018; Parejo-Pulido et al. 2023) or not manipulated at all (Maronde and Richner 2015) on the day of observation. A possible alternative explanation for the positive results found in this and Noguera et al. (2010) studies is that vitamin E alleviated the inhibitory effects on begging caused by an acute stress response following experimental manipulation. (Wada and Breuner 2008; Tilgar et al. 2010). It is long known that stressful stimuli triggering a fear response in chicks mediated by the hypothalamic–pituitary–adrenal (HPA) axis can inhibit begging (Rydén 1980; Wada and Breuner 2008; Tilgar et al. 2010), particularly vocal begging, as an adaptive response to avoid detection by eavesdropping predators (Haff and Magrath 2010; Yasukawa et al. 2020). Several studies have found that vitamin E suppresses the elevation in plasma corticosterone due to pituitary ACTH in both chickens (Taniguchi et al. 2001) and rats (Nur Azlina and Nafeeza 2008; Ibrahim et al. 2012). Across many different bird species, chick nutritional condition is inversely correlated with either baseline or stress-induced glucocorticoids (Dantzer and Newman 2022). This may explain why effects of vitamin E supplementation on begging output are often restricted to the less competitive chicks, e.g. smaller gull chicks (Noguera et al. 2010) or female great tit nestlings (Maronde et al. 2018). In chicken and other birds, glucocorticoids secreted during an acute or chronic stress response increase the production of ROS and reduce plasma antioxidant capacity, shifting individuals into oxidative stress (Costantini et al. 2011; Haussmann et al. 2012). Therefore, glucocorticoids can mediate non-causal associations between chick oxidative

status and begging performance. This association can be mistakenly taken as evidence in support of the hypothesis that oxidative stress is a mechanism underlying begging behaviour. In magpie nestlings forced to beg longer than controls by experimentally withholding food (Moreno-Rueda et al. 2012), the stress of waiting for food could have induced the activation of the HPA axis (Casagrande et al. 2016). In yellow-legged gulls, *in ovo* injection of corticosterone reduces pecking (begging) behaviour in newly hatched chicks (Rubolini et al. 2006) and, when administered to older chicks, it promotes oxidative stress regardless of vitamin supplement (Kim et al. 2013).

Summarizing, despite its limitations, this study suggests that oxidative costs of begging are minor or absent in Barn Swallows and casts doubt on a putative oxidative cost of chick begging. In such case, either i) we still ignore the proximate mechanism underlying individual begging costs (Moreno-Rueda et al. 2012), or 2) signalling models of costly begging should be abandoned in favour of cheaper alternatives (Számádó 2011). Future empirical tests of this hypothesis should consider (1) multiple components of begging displays (e.g. postural, and acoustic); (2) validating the oxidative status of nestlings during begging trials; (3) other factors likely to affect begging performance (e.g. nestling sex or relative hunger); and (4) alternative explanations for an association between oxidative status, antioxidant supplementation and begging behaviour, particularly those involving treatment-induced differences in nestling growth and glucocorticoid-mediated response. Also, within-subject comparisons where begging behaviour is measured before and after treatment (appropriately controlling for age/order effects) may provide a more robust experimental design.

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Author contributions MF-R: Writing—Original Draft (supporting). AC: writing – original draft (supporting); methodology (equal). AJR: Methodology (equal). NSS: Methodology (equal). TR: Conceptualization (lead); Methodology (equal); Writing – original draft (lead).

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Data availability The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

Declarations

Conflict of interest None declared.

Ethical approval The experimental procedures were approved by the CSIC Ethical Committee and authorized by the Andalusian Competent Authority (ref. 11_16-Redondo), to comply with Spanish and European laws on the protection of animals used for scientific purposes.

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