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Research

Effects of supplemental feeding on nesting success and physiological metrics in eastern bluebirds *Sialia sialis*

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Supplemental feeding is a common anthropogenic influence on wildlife which, dependent on natural food availability, can have positive or negative effects on physiological condition. For example, animals may respond negatively to supplemental feeding if the artificial food source increases disease exposure or there may be negative consequences from removal of a supplemental food source. We manipulated supplemental food availability in a wild population of eastern bluebirds *Sialia sialis* to examine the influence on body mass, physiological metrics and nesting success. Adult and nestling bluebirds were randomly assigned to one of three feeding groups. The first treatment group received mealworm *Tenebrio molitor* larvae inside nest boxes throughout the breeding attempt, the second treatment group received mealworms from nest completion until nestlings hatched, and the third treatment group received no supplementation. We collected blood samples from adults and nestlings to quantify bacterial killing ability, corticosterone levels and heterophil to lymphocyte ratios. As measures of nesting success, we quantified hatching success and fledging success. Supplement group tended to impact nestling mass near fledging; however, neither the physiological metrics nor the nesting success metrics differed significantly among experimental groups. Our results suggest eastern bluebird supplementation is largely neutral with the exception of its removal at the time of hatching, at least when natural food sources are abundant. Bird feeding by hobbyists may attract birds to locations with available nesting sites without demonstrably negative or positive effects, unless practiced inconsistently during breeding.

Keywords: bacterial killing ability, bird feeding, corticosterone, leukocyte ratio, reproductive success



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Introduction

Abundance and timing of food availability can drive large-scale ecological patterns in species' distributions and population size (White 2008). Humans directly modify food availability through supplemental feeding (Robb et al. 2008a, Sguassero et al. 2012, Oro et al. 2013). In particular, the rapid rise of the multi-billion-dollar industry dedicated to feeding birds has contributed to dramatic shifts in community composition and phenology (Plummer et al. 2015, 2019). Supplemental feeding has increased bird population sizes and advanced laying dates in some species (Ewald and Rohwer 1982, Galbraith et al. 2015).

Benefits of providing supplemental feeding for birds have also been observed at the individual level on physiological metrics, nestling growth and nest success, a crucial fitness component. Supplemental food can increase feather growth rates, increase antioxidant levels and improve markers of condition (Robb et al. 2008a, Chamberlain et al. 2009, Wilcoxen et al. 2015). Nestlings from nests provided with supplemental food may fledge at a higher mass than nestlings in unsupplemented nests (Knutie 2020, Dzielski et al. 2021), and nestlings are overall more likely to fledge from nests provided with supplemental food (Robb et al. 2008a). Some physiological metrics may provide more sensitive and easy to measure indicators of the effects of supplemental feeding on birds at the individual level than estimates of survival (Marra and Holberton 1998).

For example, secretion of the steroid hormone corticosterone (CORT) can increase in response to low food availability or experimental fasting (Lynn et al. 2003, 2010, Kitaysky et al. 2010). Chronic elevation of CORT may be associated with reduced survival between years (Goutte et al. 2010) as well as lower immune function (Angelier et al. 2010). Animals living in lower quality habitats with lower quality diets often have higher baseline CORT levels than conspecifics living in better quality habitats (reviewed by Romero and Wingfield 2015). However, other metrics, such as the leukocyte profile, may be a better indicator of prolonged exposure to stressors (McFarlane and Curtis 1989, Maxwell 1993, Vleck et al. 2000, Mashaly et al. 2004).

The leukocyte profile of an individual, particularly the ratio of heterophils to lymphocytes (H:L), can change in response to environmental stressors (Lobato et al. 2009). Lymphocytes function in systemic pathogen defense and include T-cells, B-cells and natural killer cells (Berrington et al. 2005). Within an immunological context, lower heterophil numbers indicate that cells have not been recently triggered by an inflammatory response or foreign bacteria (Davis et al. 2008). After exposure to a stressor, the number of heterophils in circulation increases and the number of lymphocytes decreases, increasing the H:L ratio typically within 1–2 h (Davis et al. 2008). This change in the leukocyte profile occurs more slowly than the elevation of circulating CORT levels and persists for at least hours afterward in mammalian species (Davis et al. 2008). Monitoring of this metric may provide important insight into the effects of reduced food

availability over a longer duration, such as a nesting attempt. Though H:L ratios may indicate recent bacterial infections, they are not indicative of the capacity of an individual to limit infection through immunological responses, which may be best reflected in functional immune assays, such as the bacterial killing assay.

Bacterial killing ability (BKA) can indicate physiological condition and survival probability (Hornef et al. 2002) because higher BKA is reflective of resistance to bacterial infection (Tieleman et al. 2005, Millet et al. 2007, O'Neal and Ketterson 2012). BKA is particularly relevant for birds utilizing supplemental feeding sites because individuals at bird feeders may be at increased risk of exposure to diseases, such as *Salmonella*, *Avipoxvirus*, *Mycoplasma gallisepticum* and *E. coli*, especially when sites are not cleaned (Brittingham and Temple 1986, Fischer et al. 1997, Foster et al. 2006, Robb 2008b) and conspecific contact increases through aggregation at communal feeders.

We examined the potential effects of supplemental feeding on the physiology and nesting success of eastern bluebirds *Sialia sialis*. Eastern bluebirds are insectivorous passernines commonly provided with mealworm larvae *Tenebrio molitor* as a supplemental food source. We manipulated mealworm availability for adults and their nestlings by placing small cups with mealworms inside nest boxes, which ensured that the adults and nestlings using the nest box were the only birds accessing the food. By design we also isolated the effects of food supplementation from any effects of increased disease exposure from conspecifics due to congregation at feeders, which was important because we quantified immune responses in supplemented and unsupplemented birds. This design differs from previous studies in which feeders were mounted externally (Brittingham and Temple 1986, Wilcoxen et al. 2015).

Given the benefits of supplemental feeding for birds, inconsistent access to supplemental food sources or sudden loss of a supplemental food source may have negative consequences, especially if the birds are reliant on the supplemental food. However, few studies have addressed how interruptions in food supplementation impact physiological metrics or nest success (Murray et al. 2016, Cornelius Ruhs et al. 2019). To test if removal of a supplemental food source at a crucial life history stage might impact physiological metrics or nesting success, we had three treatment groups. A fully supplemented nest (FULL) received mealworms from nest completion until fledging. Partially supplemented nests (PART) received mealworms until hatching, and control nests (CONT) did not receive any mealworms but were disturbed at the same frequency. We predicted that nestlings would be more affected by supplemental feeding than adults and that mean nestling masses near fledging would be highest in the FULL group. We predicted that nestling CORT and H:L ratios would be highest in the PART group due to removal of an initially predictable supply of supplemental food when food requirements rapidly increased (i.e. hatching stage). This manipulation was meant to imitate an irregular anthropogenic feeding schedule (Wilcoxen et al. 2015). Further, we hypothesized that BKA of

nestlings would be highest for young in fully supplemented nests because supplemental feeding prior to egg-laying improves nestling immune function (Moreno et al. 2008). Finally, we predicted that adults in the fully supplemented treatment would have greater nesting success than adults with less supplementation due to increased energy availability (Robb et al. 2008b). Our study provides insight into the physiological responses of birds to supplemental feeding and the effects of the removal of supplemental food at hatching (Brittingham and Temple 1988, Harrison et al. 2010).

Material and methods

Field methods

The range of eastern bluebirds extends from the central United States to the Atlantic Coast (Sibley et al. 2014). Bluebirds prefer to nest in edge habitats, including edges created by human development, which brings them into close contact with humans (Jones et al. 2014). Eastern bluebirds are secondary cavity nesters, and breed in artificial nest cavities. For this study, nest boxes (2015: n = 187, 2016: n = 176) located along walking trails and roads in Stillwater, Oklahoma ($36^{\circ}7'18''\text{N}$ $97^{\circ}4'7''\text{W}$) were utilized to manipulate the food available to breeding eastern bluebirds. Nest boxes were checked 2–3 times weekly throughout the breeding season during March–August in 2015 and 2016. Individual boxes were monitored daily when complete nests were detected to determine first egg dates. Boxes were again monitored daily when clutches were within two days of their expected hatch date (clutch completion date + 13 d). Then nest boxes were monitored during regular nest checks to determine if fledging occurred. This frequency of monitoring generally follows methods used previously in this population (Grindstaff et al. 2012).

Experimental manipulation of food availability

Small feeding cups (2 oz, plastic) were mounted in all nest boxes prior to the breeding season. In these cups, supplemental food was provided by adding fifteen mealworms (1.909 ± 0.177 g) per individual bluebird in the nest box, including both adults and nestlings, three times per week (~11.4–40 g weekly) (Smith 2020). Each time the nest boxes were visited, it was noted whether the cups were empty or contained mealworms to track if the birds had consumed them. Any nest boxes with consistently full cups, likely neophobic individuals or recently failed nests, were removed from all analyses (9.53% of 63 boxes total for both years which received PART/FULL treatments and survived to day 5). All boxes included in the analyses consumed greater than half of all mealworms provided to them.

The experimental groups were randomly allocated to FULL (2015: n = 35, 2016: n = 22), PART (2015: n = 26, 2016: n = 20) or CONT (2015: n = 36, 2016: n = 35) treatment groups. An additional subset of boxes was included as

a control for potential effects of additional foot traffic at the boxes. The boxes in this second control group did not receive supplemental food and were visited only 2–3 times per week during regular nest monitoring. All other boxes, regardless of experimental treatment, were visited and opened the same number of times to control for any effects of disturbance. Because the two control groups did not differ significantly in any of the main effects (all $p > 0.058$), these groups were combined in the analyses described below.

Morphology

All adult birds in the study were captured for banding and weighing using a nest box trap no sooner than two days after nestlings hatched (Friedman et al. 2008). Mass for each individual was determined using either a digital balance (accuracy = 0.01 g) or Pesola (accuracy = 0.1 g). Nestlings were weighed on day 14 post-hatch (day 0 = hatch day). Nestlings were banded with an aluminum USFWS band on day 11 post-hatch, and were examined to determine sex based on plumage coloration on day 14 (Pyle 1997).

Nest success

A clutch was considered complete when the number of eggs did not increase for more than one day. Hatching success was quantified as the proportion of eggs in the clutch that hatched. Fledging success was determined as the proportion of nestlings that left the nest after the day 14 measurement. The typical time to fledging in eastern bluebirds is 17–19 d (Gowaty and Plissner 2015) with nestlings capable of weak flight at day 14. Nestlings absent from the nest after day 14 were considered successfully fledged, unless there was evidence of death or predation.

Blood sampling and laboratory methods

Blood samples (50–100 μl) were collected from all nestlings and adults within three minutes of removal from the nest box to determine CORT levels (Owen 2011). Compared to other studies on this population in which CORT has been measured, CORT levels in this study were elevated (Grindstaff et al. 2012). In this study, we collected blood samples within three minutes of removal from the nest box, rather than within three minutes of initial disturbance, as in previous studies. All blood samples were taken between 07:00 and 11:00 h to minimize variation due to circadian rhythms in hormone levels (Remage-Healey and Romero 2000). Approximately 5–10 μl of each whole blood sample was used to prepare blood smears in the field. The remainder of each whole blood sample was kept cool on ice until it was brought to the laboratory for plasma separation with a centrifuge at $1846\times g$ for seven minutes, which took place within 4 h of collection. Plasma was stored at -20°C until it was used in BKA assays, or to measure plasma CORT. Average storage length prior to BKA analysis was 158 ± 3.37 d. Storage length was not significantly related to BKA; therefore, we did

not include this as a covariate in our analyses ($t(76)=2.28$, $p=0.99$). Storage length also was not significantly related to CORT levels; therefore, we did not include this as a covariate in our analyses ($t(276)=0.055$, $p=0.956$).

Slides were stained with differential quik stain following kit instructions (Davis et al. 2008). A complete blood cell count was performed following the techniques described in 'Avian and exotic animal hematology and cytology' (Clark et al. 2009, Campbell and Ellis 2013) by an expert in white blood cell identification (IK) who was blind to the identity of the treatment groups. Heterophil to lymphocyte (H:L) ratios were calculated by dividing the total number of heterophils by the total number of lymphocytes.

Enzyme immunoassays (Corticosterone ELISA Kit ADI-901-097, Enzo Life Sciences) were used to quantify circulating plasma CORT levels. Based on previous optimization of the assay for eastern bluebirds (unpubl.), plasma samples were diluted 1:40 in 1.5% steroid displacement reagent (SDR). All samples were run in duplicate and the corticosterone standards (20 000, 4000, 800, 500, 160 and 32 pg ml⁻¹) were run in triplicate. The optical density of the plates was then read at 405 nm on a BioTek ELx808 microplate reader. Samples with intra-assay coefficients of variation (CVs) higher than 15% were re-run (average intra-assay CV: 6.25%). The average inter-assay CV was 14.57%.

Tryptic soy agar plates were made under sterile conditions and stored at 4°C the day prior to conducting BKA assays. *Escherichia coli* (ATCC no. 8739, Microbiologics) stocks and dilutions were prepared the day before assays were conducted. *E. coli* stock solutions were prepared by adding one lyophilized bacteria pellet (5.6×10^7 CFU) to 40 ml of phosphate-buffered saline, which was then incubated at 37°C for 30 min and stored at 4°C. Plasma and bacteria were incubated in carbon dioxide independent media. Additionally, 200 µl of 200 mM L-glutamine was added to the solution prior to incubation. To perform the assay, 5 µl of plasma was combined with 100 µl of the media solution and 10 µl of bacterial working solution. The bacteria and plasma were incubated together for 30 min at 41°C. The samples were then plated on agar plates and incubated for a minimum of 12 h at 37°C. Control plates were prepared in the same manner as experimental plates; however, control plates did not contain plasma. The number of colonies on each plate was counted after the 12 h incubation. Finally, the BKA of each plasma sample was quantified as the percent difference in the number of colonies on plasma treated plates compared to the number of colonies on control plates.

Statistical methods

We compared masses of nestlings on day 14 among supplement groups to determine if variation in supplemental feeding schedules affected fledging mass. We used R ver. 4.1.0 to create linear mixed-effects models from the *lme4* package to account for non-independence of nestlings and mated pairs from the same nest. NestID, a concatenate variable created

with nest box names and start dates, was included as a random effect to account for non-independence and month was included as a fixed effect to account for seasonal variation. Masses of adults were collected at the time of capture and, while we did not anticipate an effect of our experiment on adult mass, we analyzed adult body mass separately using the same methods as for nestlings.

We conducted separate analyses on adults and nestlings for CORT, H:L ratios and BKA. We again used linear mixed-effects models from the 'lme4' package to account for non-independence of nestlings and included NestID, as a random effect. Month was also included in these analyses as a fixed effect. For CORT, we evaluated 56 adults and 220 nestlings. For H:L ratios, we analyzed 48 adults and 44 nestlings, and for BKA, we analyzed 49 adults and 69 nestlings. For nestling measures of H:L ratio, we selected one nestling at random from each brood prior to data collection due to constraints on supplies and personnel. We acknowledge that nestlings might exhibit high variability in physiological traits within the same nest, but we opted to capture population-level variation, rather than within nest variation. CORT and H:L ratios were not normally distributed; therefore, we normalized these values by log₁₀ transformation (Lobato et al. 2009).

To evaluate the effect of supplemental feeding on nesting success, we compared hatching and fledging success among experimental groups with month included as a fixed effect. Finally, to assess whether adult physiology was predictive of nesting success, we also determined whether CORT, BKA or H:L ratios were associated with measures of nesting success across treatment groups. We used logistic regressions with a quasibinomial distribution for these analyses because fledging and hatching success varied between 0 and 1, with intermediate values representing portions of broods that successfully hatched or fledged. To ensure independence of measurements in instances when we had data from two adults associated with a nest, we removed one individual using a random number generator in Python ver. 3.6. Thus, only one adult was associated with success at each nest. We also included month as a factor due to the potential for reproductive success to vary during the season.

Results

Body mass

Adult masses did not differ among supplement groups ($F_{2,43.16}=0.17$, $p=0.84$; effect of month: $F_{1,42.74}=0.97$, $p=0.33$; CONT: 29.39 ± 0.27 g; PART: 29.95 ± 0.77 g; FULL: 29.49 ± 0.47 g, Fig. 1). There was a nearly significant difference in nestling body mass across supplemental feeding groups ($F_{2,94.50}=3.02$, $p=0.053$; effect of month: $F_{1,93.67}=60.86$, $p < 0.001$). Nestlings in the PART group weighed the least (CONT: 26.84 ± 0.19 g; PART: 25.61 ± 0.43 g; FULL: 26.38 ± 0.33 g; Fig. 1).

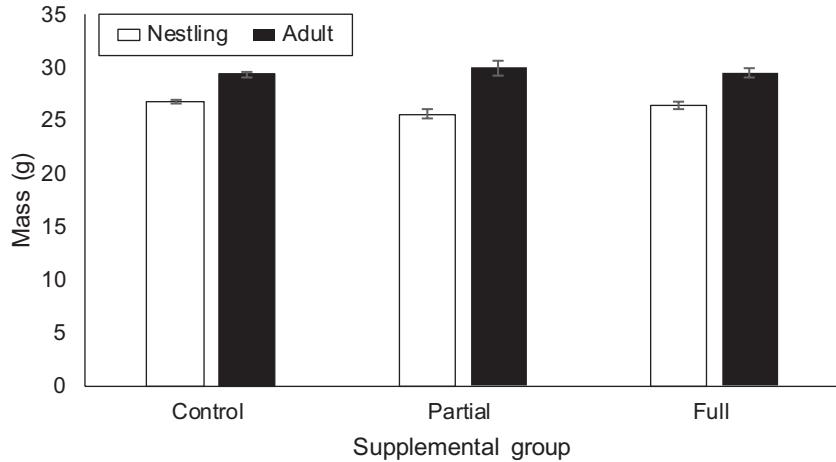


Figure 1. Mean masses of nestlings and adults in control, partially supplemented and fully supplemented groups. Error bars depict standard error in each group.

Corticosterone

There was no significant effect of supplemental feeding group on corticosterone concentrations in adults ($F_{2,36.74}=0.73$, $p=0.49$; Effect of month: $F_{1,35.37}=0.25$, $p=0.62$; CONT: $11.56 \pm 1.07 \text{ ng ml}^{-1}$; PART: 8.455 ± 1.08 ; FULL: $13.08 \pm 2.52 \text{ ng ml}^{-1}$; Fig. 2). Similarly, there was no significant effect of supplemental feeding group on corticosterone levels in nestlings ($F_{2,104.08}=0.54$, $p=0.58$; effect of month: $F_{1,99.83}=0.20$, $p=0.66$; CONT: 8.64 ± 0.43 ; PART: 9.96 ± 1.06 ; FULL: $9.02 \pm 0.74 \text{ ng ml}^{-1}$; Fig. 2).

Heterophil to lymphocyte ratios

There was no significant effect of supplement group on heterophil to lymphocyte ratios in adults ($F_{2,43}=0.20$, $p=0.82$;

effect of month: $F_{1,43}=0.26$, $p=0.61$; CONT: 0.51 ± 0.08 ; PART: 0.83 ± 0.19 ; FULL: 0.76 ± 0.21 ; Fig. 3), or nestlings ($F_{2,39}=0.22$, $p=0.80$; Effect of month: $F_{1,39}=0.51$, $p=0.48$; CONT: 0.87 ± 0.17 ; PART: 0.70 ± 0.12 ; FULL: 0.86 ± 0.19 ; Fig. 3). There were also no interactions between supplement groups, brood size and mass (all $p > 0.10$) or correlation between H:L and CORT (adults: $t(26)=-0.44$, $p=0.67$; nestlings: $t(24)=1.16$, $p=0.26$).

Bacterial killing ability

Adults in the three groups did not differ significantly in BKA ($F_{2,45}=0.42$, $p=0.66$; effect of month: $F_{1,45}=3.40$, $p=0.072$; CONT: $58.46 \pm 5.60\%$; PART: $55.83 \pm 5.98\%$; FULL: $58.98 \pm 7.15\%$; Fig. 4). There also were no significant

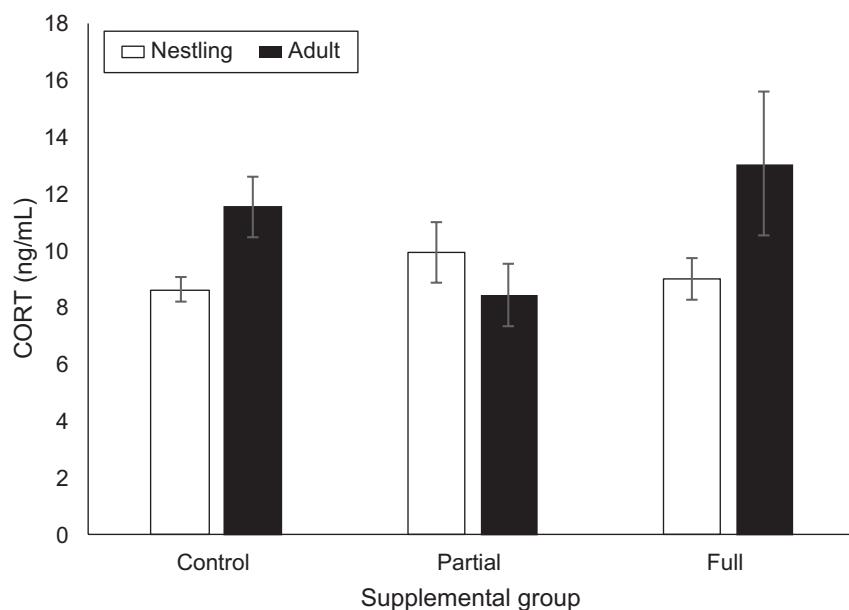


Figure 2. Mean corticosterone concentrations in ng ml^{-1} in blood plasma of adults and nestlings in the control, partially supplemented and fully supplemented groups. Standard errors are indicated by error bars.

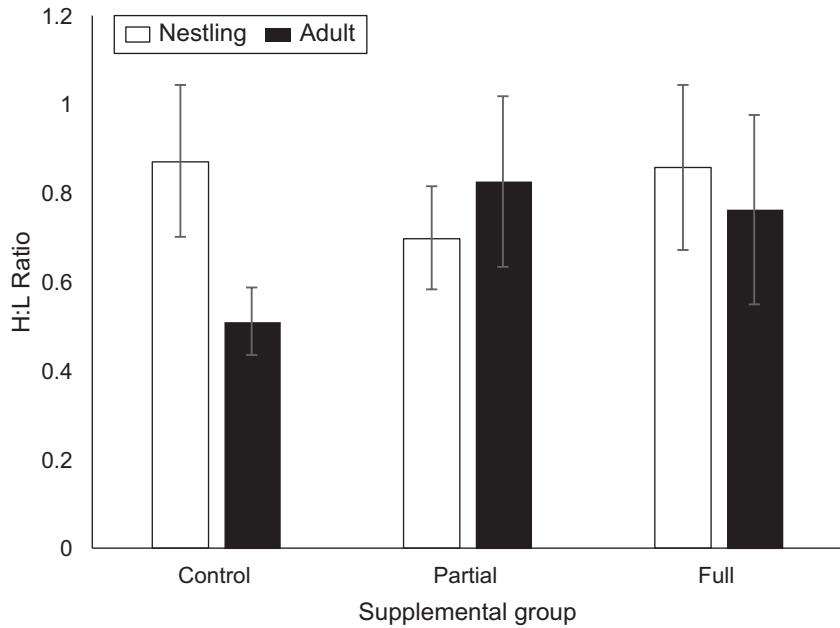


Figure 3. Mean heterophil to lymphocyte ratio across supplement groups in nestlings and adults. Error bars depict calculated standard error.

differences in BKA among nestlings in the three treatment groups ($F_{2,65}=0.76$, $p=0.47$; effect of month: $F_{1,65}=0.58$, $p=0.45$; CONT: $40.35 \pm 3.57\%$; PART: $32.87 \pm 4.92\%$; FULL: $35.41 \pm 4.14\%$; Fig. 4).

Nesting success

Clutch size did not differ significantly among the treatment groups ($F_{2,35}=0.00$, $p=1.00$; effect of month: $F_{1,35}=2.80$, $p=0.10$; CONT: 4.67 ± 0.16 ; PART: 4.67 ± 0.17 ; FULL: 4.67 ± 0.17). Hatching success was not influenced by supplemental feeding group ($F_{2,35}=0.18$, $p=0.84$; effect of month: $F_{1,35}=0.23$, $p=0.63$; CONT: 0.82 ± 0.05 ; PART: 0.87 ± 0.07 ; FULL: 0.87 ± 0.07). Brood size did not

differ significantly among the treatment groups ($F_{2,35}=0.28$, $p=0.76$; effect of month: $F_{1,35}=1.56$, $p=0.22$; CONT: 3.76 ± 0.23 ; PART: 4.00 ± 0.29 ; FULL: 4.00 ± 0.33). Finally, fledging success was not influenced by supplemental feeding ($F_{2,35}=0.99$, $p=0.38$; Effect of month: $F_{1,35}=3.26$, $p=0.08$; CONT: 0.86 ± 0.07 ; PART: 0.84 ± 0.11 ; FULL: 1.00 ± 0.0 ; Fig. 5).

We tested for interactions between our physiological and nest success metrics across supplement groups and found that adult BKA was significantly and negatively associated with fledging success ($t(16)=-2.79$, $p=0.013$), indicating that adults with a higher BKA had nests with lower fledging success. Adult BKA was not associated with other metrics of nesting success (all $p > 0.15$), and neither CORT nor H:L

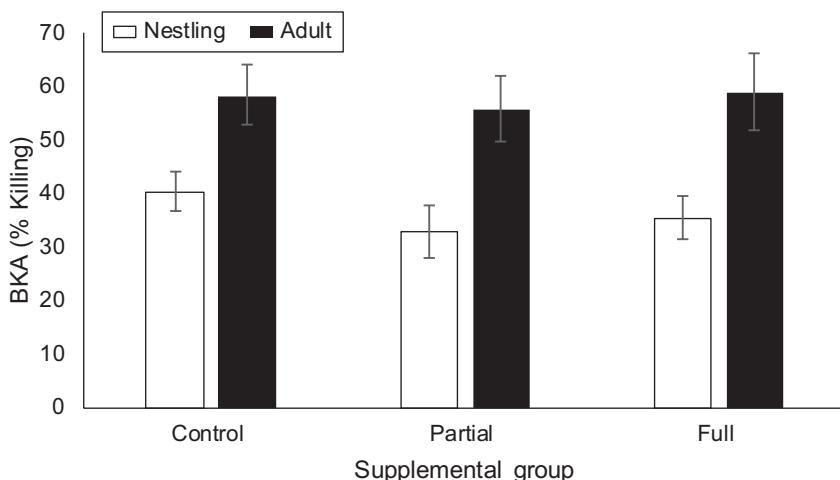


Figure 4. Mean percent BKA in nestlings and adults from control, partially supplemented and fully supplemented groups. Error bars depict standard error of each group.

ratios of adults were associated with any measures of reproductive success (all $p > 0.28$).

Discussion

Supplemental feeding did not significantly influence corticosterone levels, bactericidal capacity, heterophil to lymphocyte ratios or nest success but the removal of supplemental food had a nearly significant effect on nestling mass near fledging. These results with the exception of mass, did not support our predictions that: 1) nestlings should have increased CORT and H:L in the PART group, 2) individuals in nest boxes provided with supplemental food throughout the nesting attempt should have increased BKA and 3) that nest success would be greatest in the fully supplemented group. The lower mean nestling mass in the PART group near fledging may indicate a detrimental effect of inconsistent supplemental feeding. If parents are unable to cope with changes in environmental condition post-hatching, then nestling mass may suffer (Quinney et al. 1986). Mass at fledging may predict recruitment rates as adults, thus reduced mass may lower recruitment (Schwagmeyer and Mock 2008). We did not monitor recruitment in our study; however, it may be valuable for future studies to determine if individuals in this species have variable recruitment due to supplemental feeding.

Regarding nest success, our prediction that access to supplemental food should increase hatching success and fledging success was not supported in this study as neither of these metrics were significantly influenced by our food supplementation. Logistic regression analyses suggest that adults may trade off BKA during rearing to better care for nestlings, which has been suggested in other wild bird species (Gustafsson et al. 1994). We may not have found an effect of supplemental feeding on CORT levels in our field study

because of its sensitivity to other aspects of environmental variation, including weather events, pollution and predation (Wingfield et al. 1995, Martin 2009, O'Neal and Ketterson 2012). We did note a high CORT level in the adults. Ecological variables such as natural food availability at a site and variation in the amount of time that elapsed between disturbance of the nest and blood sample collection may explain the high CORT levels.

Heterophil to lymphocyte ratios may also have been influenced by environmental changes, as this metric is modulated by CORT (Davis et al. 2008, Dhabhar 2009), though H:L and CORT were not associated in our study. Other studies conducted on free-living birds have also not detected a relationship between H:L ratio and CORT. For example, H:L was not correlated with CORT in kestrel nestlings *Falco tinnunculus* and H:L ratios were found to be more sensitive to environmental stressors than baseline CORT (Müller et al. 2011). Further, H:L has been negatively associated with nestling growth (Moreno et al. 2002). With regard to diet and nutritional condition, research suggests that CORT may not be a reliable indicator of stress exposure related to food limitation in free-living bird species (Cyr and Romero 2007, Fairhurst et al. 2013), and H:L is a better indicator or secondary metric to include in supplementary feeding studies.

It is also possible that the birds' natural foraging behavior was not offset completely by experimental food manipulation. The North American Bluebird Society recommends providing fifteen mealworms per bluebird per day to sustain adults and nestlings (Smith 2020). Other supplementary feeding studies have provided bluebirds with 15 mealworms per nestling per day (Knutie 2020) or 10–15 g of mealworms per day at each nest box (Doyle and Siefferman 2014, Dzielski et al. 2021). Depending on the number of adults

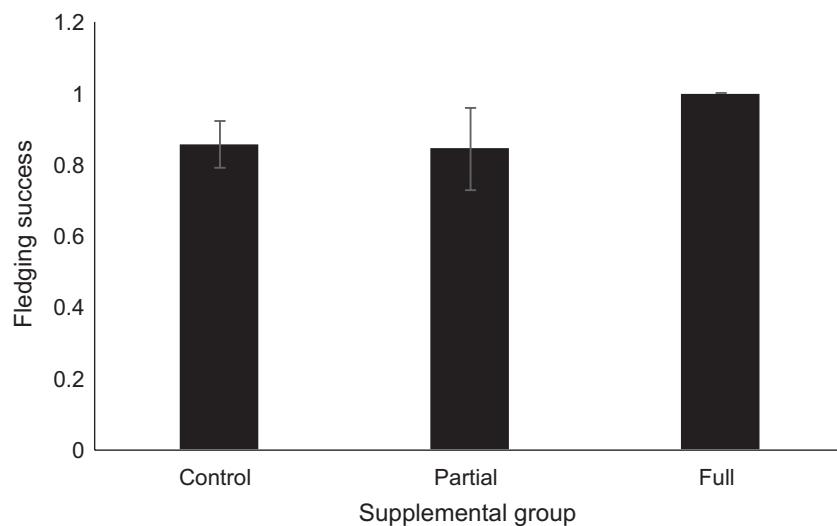


Figure 5. Average fledging success in the control, partially supplemented and fully supplemented groups. Error bars indicate the standard error of the means in each group.

and nestlings present, we provided 30–120 mealworms in each box once a day ($3.8\text{--}13.3 \text{ g d}^{-1}$), three days a week rather than daily. The daily energy requirement of adult western bluebirds *Sialia mexicana* is 95 kJ d^{-1} (Mock 1991), which translates to a demand for 22.6 kcal d^{-1} . Nestling eastern bluebirds have a daily energy requirement of 58 kJ d^{-1} by day 14 (Droge et al. 1991), which translates to a demand for 13.8 kcal d^{-1} . Mealworms contain 2.23 kcal g^{-1} (Finke 2002). Thus, the supplement we provided contained an additional 4.26 kcal for each bird associated with the nest box. This would be 18.8% of daily energetic requirements for adults and 30.9% of daily energetic requirements for nestlings near fledging. This level of supplementation may have been insubstantial compared to the birds' typical food intake needs. Further, as seen in previous studies, an abundance of food sources reduces potential food strain to the birds and presumably increases survival, but additional food is only beneficial when there is a lack of food in the environment (Schoech et al. 2007).

Most research on the effects of supplemental bird feeding has been conducted on seed eating birds. As an example, Wilcoxen et al. (2015) evaluated the effects of supplemental feeding on a variety of seed eating birds and found some improvement in the health of individuals that used supplemental feeders, including faster feather growth and increased anti-oxidant levels. Future studies of supplemental feeding in insectivorous birds could address utility of supplemental food sources. Placement (Brittingham and Temple 1988, Redpath et al. 2001) can be improved and addition of a calcium source to mealworms, which are naturally low in calcium, may increase the nutritional value of the food source for bluebirds (Smith 2020). RFID-enabled feeders might also be used to track the number of mealworms each bird is able to consume.

One potential negative effect of utilizing supplemental food sources for birds is increased exposure to disease (Lawson et al. 2018). Birds in areas with supplemental feeders are more likely to be infected with diseases such as *Mycoplasma conjunctivitis* and *Avipoxvirus*, and infected birds are not always in better physiological condition than non-supplemented birds (Wilcoxen et al. 2015). Our method of providing supplemental food possibly reduced the risk of exposure to disease by limiting aggregation near supplemental food sources. In doing so, we isolated the effects of supplemental feeding from disease exposure associated with clustering around feeders and found that supplemental feeding did not influence immune responses. Supplemental food provided in the open may provide a better representation of normal foraging behavior but does not easily allow researchers to track the amount of supplemental food consumed or to track unmarked individuals utilizing the food source, both of which are important to know when evaluating disease susceptibility.

Our study aimed to emulate supplemental feeding practiced by hobbyists. While we did not find evidence that supplemental feeding benefitted bluebirds, there also were few detrimental effects. Thus, there may be a net beneficial

effect for birds given the added benefit of engaging the public in the outdoors (Newsome and Rodger 2008). As we observed while conducting this study, members of the public are often eager to contribute to data collection and study of wild birds (Irwin 2018, Richter et al. 2018). The greater public understanding that comes with participation in science can increase public support for future scientific studies and creates a sense of environmental stewardship (Dickinson et al. 2012).

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Author contributions

Danielle Perryman: Conceptualization (equal); Data curation (equal); Formal analysis (equal); Funding acquisition (equal); Investigation (equal); Methodology (equal); Project administration (equal); Resources (equal); Writing – original draft (equal); Writing – review and editing (equal). **Meelyn Pandit:** Investigation (equal); Methodology (equal); Writing – review and editing (supporting). **Eric Riddell:** Formal analysis (equal); Visualization (supporting). **Tiana Sanders:** Investigation (equal); Methodology (equal). **Ian Kanda:** Investigation (equal); Resources (supporting). **Jennifer Grindstaff:** Conceptualization (equal); Formal analysis (equal); Investigation (equal); Methodology (equal); Project administration (equal); Resources (equal); Supervision (equal); Writing – review and editing (equal).

Transparent Peer Review

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Data availability statement

Data are available from the Dryad Digital Repository: <<https://doi.org/10.6078/D1142Q>> (Perryman et al. 2022).

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