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# Oxidative stress, circulating antioxidants, and dietary preferences in songbirds

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ABSTRACT

Oxidative stress is an unavoidable consequence of metabolism and increases during intensive exercise. This is especially problematic for migratory birds that metabolize fat to fuel long-distance flight. Birds can mitigate damage by increasing endogenous antioxidants (e.g. uric acid) or by consuming dietary antioxidants (e.g. tocopherol). During flight, birds may increase protein catabolism of lean tissue which may increase circulating uric acid and many birds also consume an antioxidant-rich frugivorous diet during autumn migration. We evaluated three related hypotheses in a migratory passerine: (1) protein consumption is positively related to circulating antioxidants, (2) a dietary oxidative stressor [i.e. polyunsaturated fatty acid (PUFA)] influences antioxidant capacity and oxidative damage, and (3) oxidative stress influences dietary antioxidant preferences. White-throated Sparrows (*Zonotrichia albicollis*) consuming a high protein diet increased circulating uric acid; however, uric acid, antioxidant capacity, and oxidative stress did not differ between birds consuming a high PUFA versus a low PUFA diet, despite increased oxidative damage in high PUFA birds. Birds did not prefer antioxidant-rich diets even when fed high PUFA, low protein. We conclude that White-throated Sparrows successfully mitigated oxidative damage associated with a high PUFA diet and mounted an endogenous antioxidant response independent of uric acid, other circulating antioxidants, and dietary antioxidants.



## 1. Introduction

All aerobic organisms produce reactive oxygen species (ROS) as unavoidable byproducts of respiration. Though some free radicals originate from exogenous sources, the most abundant ROS are produced in mitochondria in the electron transport chain (Balaban et al., 2005; Halliwell and Gutteridge, 2007). Once a free radical has participated in an oxidative reaction, it has the potential to initiate an oxidation cascade which, if left unchecked, can cause extensive damage to proteins, lipids, and DNA, ultimately leading to increased oxidative stress (Halliwell and Gutteridge, 2007). The detrimental effects of ROS on biological systems have far-reaching consequences including impaired cellular function, increased cellular senescence, and the onset of disease (Barja, 2000; Halliwell and Gutteridge, 2007).

Ecological interest in oxidative balance is largely concerned with understanding the links between oxidative status and fitness based on the assumption that poor control of oxidative stress leads to poor health (Cohen et al., 2010). Migratory birds, in particular, have become the focus of oxidative stress research due to the especially high levels of ROS production they may experience as a consequence of long-duration flight (Costantini et al., 2007, 2008). To fuel such flights, birds primarily derive energy from lipid stores accumulated prior to migration (Berthold, 2001), the majority of which are unsaturated fatty acids that are classified either as monounsaturated (MUFA) or polyunsaturated (PUFA) (Klasing, 1998; McWilliams et al., 2004). PUFA are more highly susceptible to attack by ROS due to an abundance of double bonds in their structure (Klasing, 1998; Hulbert et al., 2007) and shorter-chain PUFA are more easily metabolized than longer-chain PUFA (Price et al., 2008). Dietary fatty acids can be directly incorporated into the fat stores of migratory birds (Pierce et al., 2005) and high PUFA diets have been shown to increase oxidative stress in other species (Sies et al., 2005). As a result, high levels of ROS production may be particularly problematic for birds using lipids, and especially short-chain PUFA, to fuel long-distance fasting flights (Bairlein and Gwinner, 1994; Surai, 2002; Pierce and McWilliams, 2005).

In order to cope with the damaging effects of ROS, birds and other vertebrates have evolved sophisticated multi-level antioxidant defense systems that function to protect against free radical damage. The first level of protection minimizes uncontrolled ROS release within cells either through membrane composition or by increased uncoupling of oxygen consumption and ATP generation (Brand, 2000; Balaban et al., 2005; Hulbert et al., 2007; Monaghan et al., 2009). The second level consists of antioxidant enzymes (e.g. catalase, glutathione peroxidase, superoxide dismutase) and metal-binding proteins, which are located in the cell and act to counteract the effects of ROS (Balaban et al., 2005; Monaghan et al., 2009). Enzymatic antioxidants (e.g. superoxide dismutase, catalase) work primarily within mitochondria at the site of free radical production and are unchanged when they react with free radicals. Chain breaking antioxidants make up the next level of defense and include endogenous antioxidants produced in vivo (e.g. ubiquinone, glutathione, vitamin C, and uric acid) and dietary antioxidants (e.g. vitamin E and carotenoids) (Surai, 2002; Tsahar et al., 2006; Catoni et al., 2008b; Monaghan et al.,

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2009). Molecular antioxidants (e.g. uric acid, vitamin E) function in tissues and the bloodstream and are oxidized when reacting with free radicals. Thus, these antioxidants must be regenerated by co-antioxidants or they are lost (Cohen et al., 2008). The last level of defense is comprised of repair mechanisms that facilitate the removal or repair of damaged DNA, proteins, and lipids (Surai, 2002; Halliwell and Gutteridge, 2007; Monaghan et al., 2009).

Uric acid is the main nitrogenous waste product of uricotelic birds and is derived from both dietary (exogenous) and endogenous protein catabolism (Wright, 1995; Stevens, 1996). Recent research has provided evidence for its role as a potent antioxidant in birds. Tsahar et al. (2006) observed a positive linear relationship between uric acid and its oxidative product, allantoin, in White-crowned Sparrows (*Zonotrichia leucophrys*) during intense exercise. This relationship indicates a constant oxidation of uric acid in plasma and provides support for its function as an antioxidant. In addition, circulating uric acid levels reduced oxidative stress in a dose-dependent way in broiler chickens (Stinefelt, 2005) and serum uric acid was strongly correlated with antioxidant capacity in 95 bird species (Cohen et al., 2007, 2008, 2009). Bolser (2010) also found increased plasma uric acid in Hermit Thrushes (*Catharus guttatus*) consuming high levels of dietary protein, but no increases in birds consuming low protein. This suggests that protein catabolism may directly influence uric acid production. Though birds primarily derive energy from fat stores during long-distance fasting flight, catabolism of lean tissue also occurs, especially under certain environmental conditions (Gerson and Guglielmo, 2011). Lean tissue catabolism may thus cause circulating uric acid to increase which may, in turn, help safeguard birds against the oxidative damage associated with lipid metabolism.

Vitamin E is a lipid-soluble antioxidant which can only be obtained through the diet and its role in defending against oxidative damage in birds has been investigated in several studies. Eggs and chicks from hens receiving vitamin E supplementation had improved oxidative status, including higher levels of antioxidant enzymes (Surai, 2000), and supplementation in chickens increased uric acid and overall antioxidant status (Cohen et al., 2007). In addition, free-living birds at a stopover site in southern New England selected fruits with high levels of vitamin E (e.g. *Viburnum* spp.) over fruits with low levels of vitamin E (e.g. *Aronia* spp.) during autumn migration (Alan, 2012). Taken together, these data suggest that vitamin E is an important dietary antioxidant for birds and that it may have a synergistic relationship with other antioxidants, such as uric acid.

No previous studies have investigated the extent to which dietary protein intake directly affects levels of circulating antioxidants other than uric acid or how oxidative stress might influence these effects. In addition, none of the current research addresses the extent to which migratory songbirds prefer antioxidant-rich foods when exposed to oxidative damage. As such, we tested three related hypotheses: (1) the amount of dietary protein consumed by birds is positively related to levels of circulating antioxidants, (2) a dietary oxidative stressor (i.e. a high PUFA diet) affects antioxidant capacity, oxidative damage, and overall oxidative stress, and (3) exposure to an oxidative stressor influences the dietary antioxidant preferences of migratory passerines. We predicted that White-throated Sparrows (*Zonotrichia albicollis*) consuming more dietary protein would have higher levels of circulating uric acid and, after exposure to a dietary oxidative stressor, would have lower levels of oxidative stress than birds consuming less dietary protein. Birds consuming low levels of dietary protein and an oxidative stressor were expected to show a stronger preference for a diet supplemented with antioxidants than birds consuming more dietary protein.

## 2. Materials and methods

### 2.1. Capture methods and acclimation

Twenty juvenile White-throated Sparrows (*Z. albicollis*) were captured using mist nets (12 m long, 30 mm mesh) between 13 and 21 November 2010 at East Farm in Kingston, Rhode Island (41°28′N, 71°31′W). The birds were maintained in an indoor facility at the University of Rhode Island where they were housed individually in stainless steel cages (59 cm × 45 cm × 36 cm) in a room kept at a constant temperature (23 °C) and daily light cycle (12 h dark:12 h light; lights on at 06:00 h). All birds were fed a semi-synthetic acclimation diet that has been used successfully in previous studies (Pierce and McWilliams, 2005; Bolser, 2010) (Tables 1 and 2) and were maintained ad libitum on this diet for one month prior to beginning any dietary treatments. Fresh food and water were offered each day at lights on and all of the birds were weighed in order to monitor body condition. Immediately following the acclimation period, all of the birds began Phase 1 of a three-part experiment. The above-described protocols, as well as those detailed below, were approved by the University of Rhode Island Institutional Animal Care and Use Committee (Protocol No. AN 09-09-008).

### 2.2. Phase 1—dietary protein manipulation

During Phase 1, we manipulated dietary protein to determine how protein intake influences circulating levels of uric acid. To address this objective, half of the birds (n = 10) were randomly assigned a high protein diet and half (n = 10) were assigned a low protein diet (Table 1). All of the birds were maintained on their respective diets for 14 days. Each diet had similar fat composition but differing protein and carbohydrate content (Table 1) so that we could determine the effects of high versus low protein on circulating antioxidants while maintaining similar energy density between the two diets (20.6 kJ/g dry mass; calculated based on the energy density of non-fiber carbohydrate (17.6 kJ/g), protein (17.8 kJ/g), and fat (39.3 kJ/g); Schmidt-Nielsen, 1997).

### 2.3. Phase 2—fatty acid manipulation

During Phase 2, we investigated how a dietary oxidative stressor affects circulating antioxidants, oxidative damage, and overall oxidative stress. Half of the birds from each of the high and low protein groups from Phase 1 were presented with a dietary oxidative stressor in the form of a high PUFA oil mixture while the other birds in each dietary protein group continued to consume a low PUFA oil mixture (Tables 1 and 2). This resulted in four distinct experimental groups: high protein, low PUFA; high protein, high PUFA; low protein, low PUFA; and low protein, high PUFA (n = 5, in all cases). We added small amounts of crushed mealworms and increased the amount of amino acid mixture in all diets during Phase 2 (Table 1) because, during Phase 1, many of the birds exhibited a marked decrease in body mass over time.

As in Phase 1, the birds were maintained on the new experimental diets for 14 days in Phase 2. For the first nine days, 30 g (wet mass) of food was presented in a single ceramic dish placed in the center of each bird's cage. During the last five days of this two-week period, food was presented in two separate ceramic dishes, each containing 30 g (wet mass) of food, with one dish placed on the left side of the cage and one dish placed on the right. Each day, fresh food was offered at 06:00 h, withdrawn at 16:00 h, and weighed to determine the amount of food consumed per dish. Control dishes for each diet were also placed out in the room at 06:00 h and weighed at 16:00 h so that we could correct for mass loss due to natural dehydration in our estimates of wet intake. These measurements during the last five days of Phase 2 allowed us to establish whether or not birds exhibited an inherent cage side preference prior to beginning an antioxidant preference trial in Phase 3.

### 2.4. Phase 3—antioxidant preference trial

During Phase 3, we determined the extent to which consumption of a high PUFA diet influenced the diet preferences of birds for vitamin E, a common dietary antioxidant. For five consecutive days, we

**Table 1**
Composition of diets fed to White-throated Sparrows during a one month acclimation period and during Phases 1–3 of a three-part experiment conducted during Nov 2010–Apr 2011.

| Ingredients | Acclimation | | Phase 1 | | | | Phases 2 & 3 | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | High protein | | Low protein | | High protein | | Low protein | |
| | % Wet mass | % Dry mass | % Wet mass | % Dry mass | % Wet mass | % Dry mass | % Wet mass | % Dry mass | % Wet mass | % Dry mass |
| Casein[a] | 5.72 | 19.07 | 14.77 | 49.23 | 2.53 | 8.44 | 11.83 | 38.93 | 1.83 | 6.10 |
| Oil[b] | 7.40 | 24.67 | 7.40 | 24.67 | 7.40 | 24.67 | 7.40 | 24.67 | 7.40 | 24.67 |
| D-glucose[c] | 12.64 | 42.13 | 3.00 | 10.00 | 14.10 | 47.00 | 3.00 | 10.00 | 12.05 | 40.16 |
| Cellulose[d] | 1.41 | 4.70 | 1.93 | 6.42 | 2.88 | 9.60 | 1.93 | 6.42 | 2.88 | 9.60 |
| Amino acid mix[e] | 0.08 | 0.27 | 0.05 | 0.18 | 0.24 | 0.79 | 0.45 | 1.50 | 0.45 | 1.50 |
| Salt mix[f] | 1.25 | 4.17 | 1.25 | 4.17 | 1.25 | 4.17 | 1.25 | 4.17 | 1.25 | 4.17 |
| Vitamin mix[g] | 0.25 | 0.83 | 0.25 | 0.83 | 0.25 | 0.83 | 0.25 | 0.83 | 0.25 | 0.83 |
| Agar[h] | 1.25 | 4.17 | 1.25 | 4.17 | 1.25 | 4.17 | 1.25 | 4.17 | 1.25 | 4.17 |
| Mealworms[i] | – | – | – | – | – | – | 2.64 | 8.80 | 2.64 | 8.80 |
| Water | 70.00 | – | 70.00 | – | 70.00 | – | 70.00 | – | 70.00 | – |
| Vitamin E (IU/kg)[j] | 175 | 175 | 175 | 175 | 175 | 175 | 175 or 675 | 175 or 675 | 175 or 675 | 175 or 675 |

[a] Protein: High N Casein, USB Corp., Cleveland, OH, USA.
[b] Fat: 95% Canola Oil, Stop and Shop Brand, Foodhold USA, LLC, and 5% Flaxseed Oil, Jedwards International, Inc., Quincy, MA, USA, were used for all birds in Phase 1 (n = 20) and for half of the birds in Phases 2 and 3 (n = 10). A mixture of 95% Flaxseed Oil and 5% Canola Oil was used for the other half of the birds in Phases 2 and 3 (n = 10).
[c] Soluble carbohydrate: Fisher Scientific, Pittsburgh, PA, USA.
[d] Complex carbohydrate: Celufil, USB Corp., Cleveland, OH, USA.
[e] Composition of this amino acid mix (Murphy and King, 1982) plus casein satisfies the maintenance requirements of White-crowned Sparrows (Murphy, 1993); all amino acids supplied by Fisher Scientific, Pittsburgh, PA, USA.
[f] Briggs-N salt mixture: MP Biomedicals, Inc., Irvine, CA, USA.
[g] AIN-76 Vitamin and minerals mix: MP Biomedicals, Inc., Irvine, CA, USA.
[h] Agar bacteriological grade: USB Corp., Cleveland, OH, USA.
[i] Freeze-dried mealworms: Exotic Nutrition, Newport News, VA, USA.
[j] Baseline vitamin E content of each diet was 175 IU/kg. Additional vitamin E was added to one dish per bird in Phase 3 to yield a total of 675 IU/kg: MP Biomedicals, Inc., Irvine, CA, USA.

offered each bird 30 g of food in each of two separate ceramic dishes, one on each side of the cage, at lights on. Control plates for each diet were also placed out in the room at the same time to account for dehydration. All dishes were withdrawn at 16:00 h and weighed. One dish in each cage was supplemented with a high level of vitamin E (500 IU/kg) while the other remained unsupplemented. Each of the diets contained a baseline level of 175 IU/kg of vitamin E due to naturally occurring amounts of this vitamin in the plant oils we used; thus, the supplemented diet contained a total of 675 IU/kg and the unsupplemented diet contained only the baseline amount (Table 1). Addition of vitamin E to the diets did not change their consistency or physical appearance from that of the unsupplemented diet. Because none of the diet groups exhibited a significant cage side preference during Phase 2, we randomly assigned diets to cage side such that half of the birds in each experimental group received their vitamin E supplemented dish on the left and half received it on the right. This placement remained consistent throughout the duration of the preference trial.

**Table 2**
Fatty acid composition of high and low PUFA diets fed to White-throated Sparrows during Phases 1–3 of a three-part experiment conducted from Nov 2010 to Apr 2011.

| Fatty acid[a] | % Fatty acid composition[b] | | |
|---|---|---|---|
| | Acclimation and Phase 1[c] | Phases 2 and 3[d] | |
| | Low PUFA[e] | Low PUFA[e] | High PUFA[f] |
| 10:0 | – | – | – |
| 16:0 | 1.09 | 3.16 | 3.38 |
| 16:1 | 0.05 | 0.05 | 0.002 |
| 18:0 | 0.57 | 0.69 | 1.11 |
| 18:1 | 14.70 | 18.64 | 9.44 |
| 18:2 | 4.61 | 6.73 | 5.33 |
| 18:3 | 2.79 | 2.92 | 12.74 |
| 20:0 | 0.17 | 0.17 | 0.01 |
| 20:1 | 0.30 | 0.30 | 0.02 |
| 22:0 | 0.07 | 0.07 | 0.005 |
| Other[g] | 0.35 | 0.76 | 1.45 |
| Total PUFA | 7.40 | 9.65 | 18.07 |
| Total MUFA | 15.10 | 19.03 | 9.90 |

[a] Fatty acid nomenclature = C:D where C refers to the number of carbon atoms in the chain and D refers to the number of double bonds present.
[b] Calculated based on Fast (1970) and USDA (2011).
[c] Does not contain mealworms.
[d] Contains mealworms.
[e] 95% Canola Oil; 5% Flaxseed Oil.
[f] 5% Canola Oil; 95% Flaxseed Oil.
[g] Other fatty acids than those listed.

### 2.5. Blood sampling and plasma analysis

A total of four blood samples were taken from each bird over the course of the study and included a baseline sample (0 d), a sample following Phase 1 and prior to Phase 2 (14 d), a sample following Phase 2 and prior to Phase 3 (28 d), and a final sample directly following Phase 3 (33 d). All blood samples were taken at 16:00 h to ensure that the birds were in a fed state. To minimize the stress response, only as many birds (n = 6–8) as could be bled within 10 min of entry into the room were sampled on a given day. Blood sampling was thus staggered over a series of three days for each of the four blood samples taken throughout the experiment. Between 50 and 150 μL of blood were collected from each bird into heparinized capillary tubes (70 μL heparinized micro-hematocrit capillary tubes, Fisher Scientific, Pittsburg, PA, USA) via brachial venipuncture. Within 20 min of sampling, the blood was centrifuged at 12,000 *g* (Haematokrit 20, Andreas Hettich GmbH & Co. KG, Tuttlingen, Germany) for 6 min and the resulting plasma was collected and stored at −80 °C for later analysis.

We chose to use commercial kits adjusted for small sample volume to measure circulating uric acid (TECO Diagnostics, Anaheim, CA, USA; Smith et al., 2007) and oxidative damage (dROMs) (Diacron International, Grosseto, Italy; Costantini et al., 2007). There are currently several methods available to assess plasma antioxidant capacity, the benefits and drawbacks of which have been extensively debated and reviewed (Cohen et al., 2007; Costantini et al., 2007; Monaghan et al., 2009; Costantini, 2011); however, two of the more commonly used assays in avian research are the trolox-equivalent antioxidant capacity (TEAC) assay (Cohen et al., 2007) and the OXYAdsorbent (OXY) Test (Costantini et al., 2007, 2008; Costantini, 2010, 2011). For the purposes of comparing these two measures of antioxidant capacity, we used a modified version of the TEAC assay (Miller et al., 1993) following the protocol described by Cohen et al. (2007) as well as the OXY

commercial kit (Diacron International, Grosseto, Italy; Costantini et al., 2007). All plasma samples were analyzed using a microplate spectrophotometer (Bio-TekPowerwave X340, Bio-Tek Instruments, Inc., Winooski, VT, USA) and 96-well microplates.

An overall measure of oxidative stress (OS) was calculated by taking the ratio between dROMs and OXY and multiplying it by 10 (OS = dROMs:OXY × 10) as specified by Costantini et al. (2007).

### 2.6. Statistical analysis

#### 2.6.1. Normality and homogeneity of variance

All of the dependent variables (Body Mass, UA, OXY, TEAC, dROMs, OS, cage side consumption difference, vitamin E consumption difference) were tested in R (R Core Development Team, 2009) for normality using Shapiro–Wilks tests and through inspection of Q–Q plots. Homogeneity of variance was assessed using Levene's tests (R). UA and TEAC were not normally distributed and were natural log-transformed to meet these assumptions as specified by Cohen et al. (2007). All subsequent reference to UA and TEAC in the analysis refers to the transformed values unless otherwise noted.

#### 2.6.2. Liner regression and correlations

A simple linear regression analysis was conducted in SAS (SAS 9.2, SAS Institute, 2009) to assess the relationship between UA and TEAC. Other studies using these two measures found that UA is a large component of circulating antioxidant capacity and thus makes up the majority of the antioxidants measured by TEAC (Cohen et al., 2007; Bolser, 2010). As such, residuals were generated to represent the portion of TEAC not accounted for by UA (resTEAC). Pearson's Product–Moment Correlations were estimated in SAS for each pair of seven dependent variables (Body Mass, UA, OXY, TEAC, resTEAC, dROMs, and OS) for each of the three phases. Correlations were not conducted between OS and OXY or dROMs since OS is a ratio of the two and is thus not independent from these measures.

#### 2.6.3. Two-sample t-tests

Baseline values of 5 dependent variables (Body Mass, UA, OXY, dROMs, and OS) were assessed in R using two sample t-tests to determine whether there were significant differences between experimental groups before any treatments were initiated. Insufficient plasma was available to assess baseline TEAC and resTEAC.

#### 2.6.4. ANOVA models

We used a repeated-measures analysis of variance (rmANOVA) design to directly assess differences in metabolites due to dietary treatments as well as to compensate for our small sample size. Using the MIXED procedure in SAS, separate rmANOVA models were constructed for each of the dependent variables for each phase of the experiment. Variance components were estimated using the residual maximum likelihood (REML). Phase 1 included the baseline (0 d) and 14 d blood values; Phase 2 included the 14 d and 28 d values; and Phase 3 included the 28 d and final (33 d) values. Insufficient plasma was available to assess Phase 1 differences for TEAC and resTEAC. For Phase 1 we assessed the effects of protein, day, and protein∗day. For Phases 2 and 3, we assessed the effects of protein, PUFA, day, protein∗PUFA, protein∗day, PUFA∗day, and protein∗PUFA∗day. Where interaction terms were not significant, they were removed from the model in a step-wise manner with the least significant being removed first until all non-significant interaction terms were eliminated or until only the main effects remained. Day (0 d, 14 d, 28 d, 33 d) represented the repeated measure for all three phases and individual bird was specified as a random effect. We used second-order Akaike's Information Criterion ($AIC_c$) values to determine the best-fitting covariance structure for each model. A compound symmetric covariance structure was used in the following models: body mass (Phases 1–3), uric acid (Phase 3), OXY (Phase 3), dROMs (Phases 1–3), and OS (Phases 1 and 3). A heterogeneous compound symmetric covariance structure was used in the following models: uric acid (Phases 1 and 2), OXY (Phases 1 and 2), and OS (Phase 2). Finally, a variance component covariance structure was used in the following models: TEAC (Phases 2 and 3), and resTEAC (Phases 2 and 3).

It takes several days for birds to establish a diet preference if one exists (Pierce and McWilliams, 2005; Boyles, 2011). As such, cage side consumption was calculated using the average consumption difference (g wet mass) for days 4 and 5 of the trial between the left and right cage sides. Vitamin E consumption was quantified in a similar manner by calculating the average consumption difference between the supplemented and unsupplemented dishes. A two-way ANCOVA model was constructed with vitamin E consumption difference as the dependent variable and protein, PUFA, and protein∗PUFA as effects. Cage side consumption difference was included as a covariate, but no significant effect was observed. We thus reran the model as a simple two-way ANOVA with protein, PUFA, and protein∗PUFA as effects.

For all statistical analyses, effects were considered significant at the $\alpha = 0.05$ level.

## 3. Results

### 3.1. Correlations between dependent variables

We detected a significant positive linear relationship between natural log-transformed uric acid (UA) and trolox-equivalent antioxidant capacity (TEAC) ($R^2 = 0.49$, $y = 0.801x + 2.571$; $t_{45} = 0.12$, $p < 0.0001$). Body mass was positively correlated with OXY and negatively with OS in Phase 2 (Table 3). UA exhibited a positive relationship with OXY in Phase 1 (Table 3). TEAC and dROMs exhibited a consistent negative relationship with one another in both Phases 2 and 3 (Table 3) and OXY was positively correlated with dROMs in Phase 2 (Table 3). We detected no significant relationships between resTEAC and any of the other dependent variables (Table 3).

### 3.2. Differences between treatment groups

#### 3.2.1. Values of dependent variables prior to diet treatments

Baseline values for body mass, OXY, dROMs, and OS did not differ significantly between experimental groups before the start of the experiment ($t_{18} = 0.37$, $t_{16} = 1.79$, $t_{11} = 0.55$, $t_{11} = 1.67$, respectively; $p > 0.09$ in all cases); however, UA was higher for birds in the high protein group than for birds in the low protein group before any treatments were initiated ($t_{16} = 2.51$; $p = 0.02$).

#### 3.2.2. Phase 1 (0–14 days)—dietary protein manipulation

During Phase 1, body mass decreased significantly for all birds independently of diet group (protein: $F_{1,18} = 0.06$, $p = 0.81$; day: $F_{1,18} = 40.38$, $p < 0.0001$; protein∗day: $F_{1,18} = 0.44$, $p = 0.51$) (Fig. 1). Birds fed a high protein diet had higher and increased levels of UA in comparison to birds fed a low protein diet and there was little change in UA over Phase 1 for low protein birds (protein: $F_{1,16} = 11.87$, $p = 0.003$; day: $F_{1,16} = 0.41$, $p = 0.52$; protein∗day: $F_{1,16} = 4.16$, $p = 0.06$) (Fig. 2a). OXY was higher in birds consuming a high protein diet than in those consuming a low protein diet (protein: $F_{1,16} = 5.65$, $p = 0.030$; day: $F_{1,16} = 1.14$, $p = 0.30$; protein∗day: $F_{1,16} = 0.47$, $p = 0.50$) (Fig. 2d). Interestingly, dROMs increased in birds fed the high protein diet and decreased in birds fed less protein (protein: $F_{1,9} = 4.27$, $p = 0.07$; day: $F_{1,9} = 0.00$, $p = 0.97$; protein∗day: $F_{1,9} = 8.02$, $p = 0.020$) (Fig. 2e). OS was not significantly different between diet groups nor did it change from baseline levels (protein: $F_{1,9} = 0.53$, $p = 0.49$; day: $F_{1,9} = 0.00$, $p = 0.97$; protein∗day: $F_{1,9} = 1.52$, $p = 0.25$) (Fig. 2f).

#### 3.2.3. Phase 2 (14–28 days)—dietary fatty acid manipulation

For Phase 2, the rmANOVA for all dependent variables except dROMs revealed significant effects, at most, for the main effects of protein and day with no significant interactions. As such, we report the F- and

**Table 3**
Pearson Product–Moment Correlation coefficients and p-values for each pair of non-transformed dependent variables (Body Mass, UA, TEAC, resTEAC, OXY, dROMs, and OS) for Phases 1–3. Bolded text denotes significant correlations ($p<0.05$).

| | UA | TEAC | resTEAC | OS | OXY | dROMs |
|---|---|---|---|---|---|---|
| Body Mass | | | | | | |
| *Phase 1* | r = −0.071; p = 0.782 | – | – | r = −0.283; p = 0.400 | r = −0.043; p = 0.866 | r = −0.236; p = 0.485 |
| *Phase 2* | r = −0.179; p = 0.476 | r = 0.252; p = 0.514 | r = 0.316; p = 0.408 | **r = −0.614; p = 0.012** | **r = 0.476; p = 0.034** | r = −0.272; p = 0.308 |
| *Phase 3* | r = 0.105; p = 0.660 | r = 0.188; p = 0.502 | r = −0.345; p = 0.228 | r = −0.14; p = 0.552 | r = 0.100; p = 0.676 | r = −0.341; p = 0.153 |
| UA | | | | | | |
| *Phase 1* | – | – | – | r = −0.333; p = 0.317 | **r = 0.508; p = 0.031** | r = 0.148; p = 0.665 |
| *Phase 2* | – | – | r = −0.258; p = 0.503 | r = −0.031; p = 0.917 | r = 0.162; p = 0.522 | r = −0.187; p = 0.522 |
| *Phase 3* | – | – | r = −0.215; p = 0.461 | r = 0.295; p = 0.220 | r = 0.195; p = 0.411 | r = 0.352; p = 0.140 |
| TEAC | | | | | | |
| *Phase 1* | – | – | – | – | – | – |
| *Phase 2* | – | – | r = −0.295; p = 0.441 | r = −0.437; p = 0.240 | r = 0.417; p = 0.265 | **r = −0.721; p = 0.028** |
| *Phase 3* | – | – | r = 0.229; p = .431 | r = −0.533; p = 0.056 | r = 0.117; p = 0.679 | **r = −0.539; p = 0.047** |
| resTEAC | | | | | | |
| *Phase 1* | – | – | – | – | – | – |
| *Phase 2* | – | – | – | r = −0.147; p = 0.706 | r = 0.315; p = 0.408 | r = 0.453; p = 0.220 |
| *Phase 3* | – | – | – | r = −0.147; p = 0.616 | r = −0.198; p = 0.497 | r = −0.080; p = 0.786 |
| OXY | | | | | | |
| *Phase 1* | – | – | – | – | – | r = −0.121; p = 0.723 |
| *Phase 2* | – | – | – | – | – | r = 0.198; p = 0.461 |
| *Phase 3* | – | – | – | – | – | **r = 0.389; p = 0.010** |

p-values for only the main effects for body mass, UA, TEAC, resTEAC, OXY, and OS and we report the results for both the main effects and interactions for dROMs. Body mass increased for all birds regardless of protein or PUFA treatment (protein: $F_{1,16}=0.35$, $p=0.56$; PUFA: $F_{1,16}=0.01$, $p=0.96$; day: $F_{1,16}=17.94$, $p<0.001$) (Fig. 1). Birds consuming higher levels of protein continued to have higher levels of UA than birds fed less protein, and UA in birds consuming high PUFA was not different from that of birds fed low PUFA (protein: $F_{1,16}=20.25$, $p<0.001$; PUFA: $F_{1,16}=0.01$, $p=0.94$; day: $F_{1,16}=0.08$, $p=0.79$) (Fig. 2a). TEAC levels were higher in birds fed a high protein diet than in birds consuming less protein; however, TEAC levels were not different between the high and low PUFA treatment groups (protein: $F_{1,10}=7.43$, $p<0.021$; PUFA: $F_{1,10}=0.11$, $p=0.75$; day: $F_{1,10}=0.47$, $p=0.51$) (Fig. 2b). Both resTEAC and OXY were not significantly different for birds fed a high protein diet versus those fed a low protein diet, nor did they differ between high and low PUFA birds (protein: $F_{1,10}=0.51$, $p=0.49$; PUFA: $F_{1,10}=0.90$, $p=0.37$; day: $F_{1,10}=0.00$; $p=0.99$ and protein: $F_{1,16}=0.76$, $p=0.40$; PUFA: $F_{1,16}=0.02$, $p=0.90$; day: $F_{1,16}=0.14$; $p=0.71$, respectively) (Fig. 2c,d). Birds consuming a low protein diet had significantly increased dROMs levels whereas those fed a high protein diet had significantly decreased dROMs levels (protein: $F_{1,12}=8.07$, $p=0.015$; day: $F_{1,12}=8.77$, $p=0.012$; protein∗day: $F_{1,12}=16.12$, $p=0.002$) (Fig. 2e). Birds fed high PUFA diets had higher dROMs levels in comparison to birds fed low PUFA diets (PUFA: $F_{1,12}=0.05$; $p=0.83$; protein∗PUFA: $F_{1,12}=0.94$; $p=0.35$; PUFA∗day: $F_{1,12}=10.37$, $p=0.007$; protein∗PUFA∗day: $F_{1,12}=1.63$, $p=0.23$) (Fig. 2e). OS decreased during Phase 2 for all birds, independently of protein or PUFA treatment, with birds fed the high protein, low PUFA diet showing the largest decrease (protein: $F_{1,12}=1.77$, $p=0.21$; PUFA: $F_{1,12}=0.37$, $p=0.56$; day: $F_{1,12}=5.58$, $p=0.04$) (Fig. 2f).

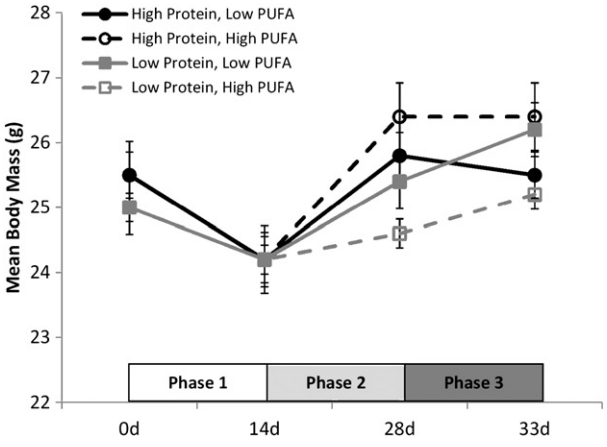


**Fig. 1.** Mean (±SEM) body mass (g) for captive White-throated Sparrows fed high protein versus low protein diets for Phases 1–3 of a three-part experiment conducted from Nov 2010 to Apr 2011. Body mass values for each bird were an average of the day before blood sampling and the day of blood sampling for each phase of the experiment.

*3.2.4. Phase 3 (28–33 days)—antioxidant preference trial*

During Phase 3, body mass increased for birds fed a low protein diet while that of birds fed a high protein diet either remained similar or slightly decreased (protein: $F_{1,16}=0.71$, $p=0.41$; PUFA: $F_{1,16}=0.01$, $p=0.92$; day: $F_{1,16}=1.53$, $p=0.23$; protein∗PUFA: $F_{1,16}=0.56$, $p=0.47$; protein∗day: $F_{1,16}=8.19$, $p=0.01$; PUFA∗day: $F_{1,16}=2.05$, $p=0.17$; protein∗PUFA∗day: $F_{1,16}=0.02$, $p=0.90$) (Fig. 1). Birds fed high protein diets continued to have higher UA levels than low protein birds, regardless of PUFA treatment, and UA levels remained consistent between sampling periods (protein: $F_{1,15}=36.56$, $p<0.0001$, PUFA: $F_{1,15}=0.01$, $p=0.92$; day: $F_{1,15}=0.01$, $p=0.92$) (Fig. 2a). TEAC did not change significantly but high protein birds continued to have higher levels of TEAC than low protein birds and this was not affected by PUFA treatment (protein: $F_{1,20}=25.14$, $p<0.0001$; PUFA: $F_{1,20}=0.04$; $p=0.85$; day: $F_{1,20}=0.82$, $p=0.38$) (Fig. 2b). Neither resTEAC nor OXY significantly increased or decreased and neither was significantly different between diet groups (protein: $F_{1,20}=0.69$, $p=0.42$; PUFA: $F_{1,20}=0.65$, $p=0.43$; day: $F_{1,20}=0.03$, $p=0.87$ and protein: $F_{1,16}=0.60$, $p=0.45$; PUFA: $F_{1,16}=0.19$, $p=0.67$; day: $F_{1,16}=0.46$, $p=0.51$, respectively) (Fig. 2c,d). dROMs and OS also did not increase or decrease significantly, nor were they different between diet groups (protein: $F_{1,15}=0.17$, $p=0.69$; PUFA: $F_{1,15}=0.40$, $p=0.54$; day: $F_{1,15}=0.08$, $p=0.78$ and protein: $F_{1,15}=0.16$, $p=0.69$; PUFA: $F_{1,15}=0.05$, $p=0.83$; day: $F_{1,15}=0.08$, $p=0.78$, respectively) (Fig. 2e,f).

When presented with a choice between a vitamin E supplemented diet and an unsupplemented diet, none of the diet groups exhibited a preference for one dish over the other (protein: $F_{1,17}=1.62$, $p=0.22$; PUFA: $F_{1,17}=0.04$, $p=0.85$) (Fig. 3).

## 4. Discussion

The main goals of our study were to: 1) investigate whether dietary protein intake was positively related to uric acid and other circulating

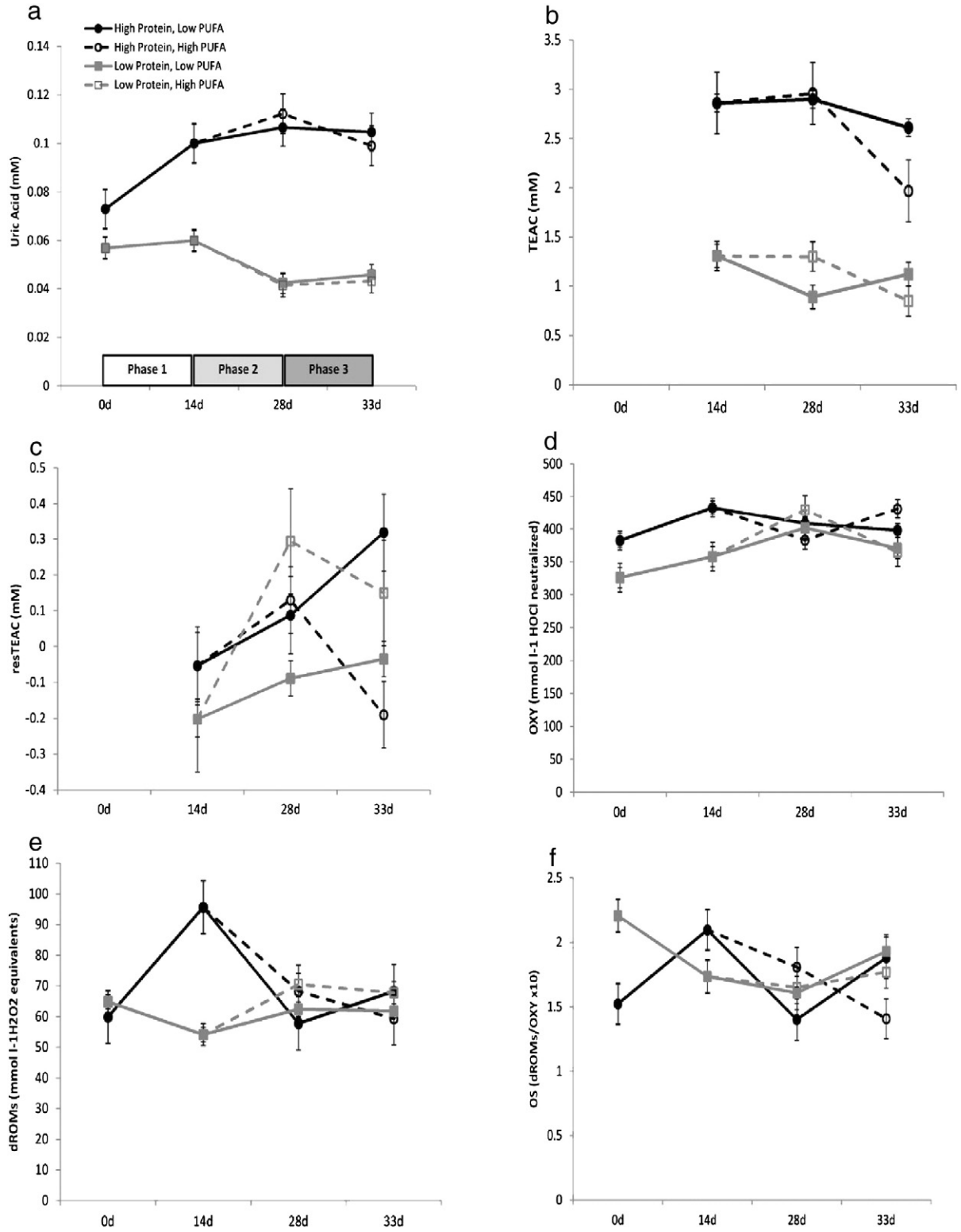


**Fig. 2.** Mean (±SEM) plasma concentration of (a) uric acid (UA), (b) trolox-equivalent antioxidant capacity (TEAC), (c) residual antioxidant capacity (resTEAC), (d) OXYAdsorbent antioxidant capacity (OXY), (e) oxidative damage (dROMs), and (f) oxidative stress (OS) for captive White-throated Sparrows fed one of four diets (high protein, low PUFA; high protein, high PUFA; low protein, low PUFA; low protein; high PUFA) during Phases 1–3 of a three-part experiment. Sufficient plasma was available to assess TEAC and resTEAC in only Phases 2 and 3.

antioxidants in a uricotelic bird, 2) determine how a dietary oxidative stressor affects antioxidant capacity and oxidative damage in a migratory passerine, and 3) determine how an oxidative stressor influenced the dietary antioxidant preferences of songbirds. We predicted that birds consuming more dietary protein would have higher levels of circulating uric acid and, after exposure to a high PUFA diet, would have lower levels of oxidative stress than birds consuming less dietary protein. We also expected that birds consuming low levels of dietary protein and a high PUFA diet would show a stronger preference for a diet supplemented with vitamin E than birds consuming more dietary protein. As outlined in

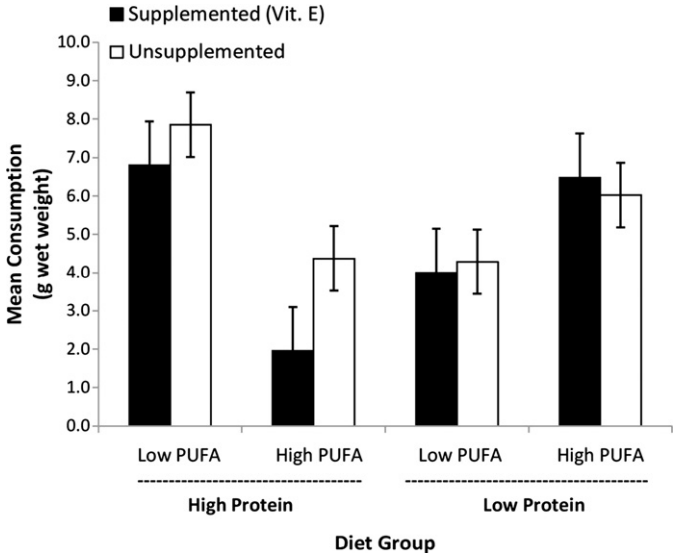


**Fig. 3.** Mean (±SEM) consumption of vitamin E supplemented and unsupplemented diets by White-throated Sparrows during the fourth and fifth days of Phase 3. Sparrows were fed one of four diets (high protein, low PUFA; high protein, high PUFA; low protein, low PUFA; low protein; high PUFA) that were presented in two dishes, one of which was supplemented with vitamin E. Sparrows consumed relatively equal amounts of the supplemented and unsupplemented diets and so did not show the expected preference for antioxidant-rich food.

more detail below, our results supported one of these three predictions and suggest that birds mounted an endogenous antioxidant response to oxidative damage independent of plasma uric acid, other circulating antioxidants, and dietary antioxidants.

### 4.1. Phase 1—dietary protein manipulation

During Phase 1, we tested the hypothesis that increased dietary protein intake was positively related to circulating uric acid and other antioxidants. As predicted, uric acid was higher in birds fed a high protein diet than in birds fed a low protein diet (Fig. 2a). These results are not novel and are consistent with the findings of similar studies in other migratory bird species which show that UA is higher in feeding birds, especially those consuming higher levels of protein (Seaman et al., 2004; Cohen et al., 2007; Smith et al., 2007; Bolser, 2010). Birds caught immediately after arriving at stopover have also been shown to have higher UA levels, which may indicate breakdown of lean tissue during long-distance flight (Landys et al., 2005). The results of these studies suggest that UA is a reliable indicator of both dietary (exogenous) and endogenous protein catabolism. High protein birds also had higher antioxidant capacity (OXY; Fig. 2d) although, contrary to expectations, they also had increased oxidative damage (dROMs; Fig. 2e) compared to low protein birds. Given that OXY does not include UA in the antioxidants it measures (Costantini et al., 2007; Costantini, 2011), it is especially interesting that OXY increased with dietary protein in the same way as UA. This corresponding increase in OXY and UA with dietary protein suggests that an increase in UA may synergistically increase levels of other circulating micromolecular antioxidants. Though this effect has not been directly demonstrated for UA, synergistic interactions between other micromolecular antioxidants have been well documented. For example, vitamins E and C are known to have a regenerative effect on carotenoids (Mortensen et al., 2001; Amorati et al., 2002; Catoni et al., 2008a) and polyphenols have a similar effect on vitamins E and C (Pietta and Simonetti, 1998; Lotito and Fraga, 2000; Catoni et al., 2008a). It is also surprising that oxidative damage (dROMs) increased with dietary protein since birds fed diets with more protein also had increased circulating antioxidants including both UA and OXY. Reactive nitrogen species, produced during protein catabolism, can cause cellular damage similar to that induced by reactive oxygen species (Surai, 2002). As such, it is possible that the increased damage we initially observed in our high protein birds was the result of increased protein catabolism caused by high dietary protein intake. Despite increased oxidative damage during Phase 1, there was no difference in OS between the diet groups, which suggests that high protein birds were able to successfully mitigate increased oxidative damage either through increased UA production or by utilizing other micromolecular circulating antioxidants.

Throughout Phase 1, all of the birds exhibited a significant decrease in body mass which we suspect was the result of an amino acid deficiency. The amino acid mixture we used has been used successfully in migratory passerines of a similar size (Murphy, 1993; Pierce and McWilliams, 2005; Bolser, 2010), so this specific mixture should also have been sufficient for our birds. Prior to beginning Phase 2, we added additional amino acid mixture to the birds' diets and the mass loss was successfully reversed (Fig. 1). This increase in weight supports our hypothesis that amino acid deficiency was responsible for Phase 1 weight loss.

### 4.2. Phase 2—dietary fatty acid manipulation

In Phase 2, we investigated the effect of a dietary oxidative stressor (i.e. a high PUFA diet) on circulating antioxidants, oxidative damage, and overall oxidative stress. As expected, birds fed a high PUFA diet had higher oxidative damage (dROMs) compared to birds fed a low PUFA diet (Fig. 2e,f). Similar effects have been observed in other animal species. For example, a higher rate of low-density lipoprotein (LDL) oxidation was observed when human subjects consumed a high PUFA diet compared to when the same subjects consumed a high MUFA diet (Bonanome et al., 1992). A diet high in PUFA was also associated with increased lipid peroxidation in rats (L'Abbè et al., 1991). The increase in oxidative damage associated with dietary PUFA that we observed in our birds was strongly affected by the amount of dietary protein, an effect that would be expected if protein catabolism provided a source of antioxidants. Though dROMs levels should have remained stable in birds consuming a low protein, low PUFA diet, these birds exhibited increased oxidative damage during Phase 2. It is difficult to definitively discern what may have caused this effect, though a wide range of variation between individual birds may have contributed. UA and TEAC remained higher for birds fed a high protein diet than for those fed a low protein diet, which was not surprising given the strong relationship between these two variables that we and others have shown (Cohen et al., 2007; Bolser, 2010) (Fig. 2a,b). In contrast, other components of antioxidant capacity, as indexed by OXY, were not affected by dietary PUFA or protein level (Fig. 2d).

Despite increased oxidative damage in birds fed a high PUFA diet, overall oxidative stress decreased in all birds regardless of protein or PUFA treatment, even in birds consuming low PUFA diets (Fig. 2f). These findings suggest that birds consuming a high protein, low PUFA diet mounted an antioxidant response to the oxidative damage incurred during Phase 1 and that all of the birds maintained on a high PUFA diet mounted an antioxidant response sufficient to mitigate the oxidative damage incurred from eating high amounts of PUFA. However, the specific antioxidant mechanisms used by birds consuming a high protein, high PUFA diet may have been different from those used by birds consuming low amounts of dietary protein. Due to the fact that oxidative damage was strongly associated with dietary protein, it is likely that UA may be used for antioxidant defense in birds consuming more dietary protein, as has been suggested in prior studies (e.g. Wright, 1995; Stevens, 1996; Tsahar et al., 2006). On the other hand, birds consuming a low protein diet were apparently equally successful at mitigating oxidative stress. Though the lack of a change in OS could simply have been the result of increased body mass during Phase 2, since OS and body mass exhibited a negative relationship with one another, these birds may also have used other components of antioxidant protection that were independent of both UA and other circulating antioxidants to mitigate oxidative damage. We suggest that some other aspects of

endogenous antioxidant capacity, such as antioxidant enzymes (e.g. catalase, glutathione, superoxide dismutase), may be upregulated in response to oxidative damage when circulating antioxidants, such as UA, are not readily available.

### 4.3. Phase 3—antioxidant preference trial

In the final phase of this experiment we investigated the effects of uric acid, antioxidant capacity, oxidative damage, and overall oxidative stress on songbird preferences for an antioxidant-rich diet. We predicted that birds fed the low protein, high PUFA diet would preferentially consume a vitamin E supplemented diet because these birds were also expected to have the highest amount of oxidative damage, lowest antioxidant capacity, and highest levels of overall oxidative stress; however, we found no differences in resTEAC, OXY, dROMs, or OS between diet groups (Fig. 2c,d,e,f). This suggests that the birds had upregulated non-circulating endogenous antioxidants to a level at which free radical production was successfully mitigated. We assumed that the effect of the high PUFA diet on oxidative damage would continue through the third phase of the experiment; however, mild exposure to oxidative stress can result in a sustained upregulation of defense levels, known as a 'hormetic effect', in which exposure to stress improves the efficacy of antioxidant protection (Rattan, 2008; Monaghan et al., 2009). It is thus not surprising that we found no effect of diet on bird preferences for foods that differed in dietary vitamin E availability because oxidative damage was likely already successfully mitigated, reducing the birds' need for dietary antioxidant supplementation (Fig. 3). Interestingly, birds from each of the four diet groups consumed different amounts of food overall (Fig. 3). Birds maintained on a high protein, low PUFA diet and on a low protein, high PUFA diet consumed more food than birds maintained on the high protein, high PUFA and low protein, low PUFA diets. This reduced food intake was not surprising for birds fed a high protein, high PUFA diet because this diet was the most nutrient-dense of the four diets used in our study and would thus allow birds to meet their daily nutrient requirements with less food. Given this reasoning, it is surprising that birds fed a low protein, low PUFA diet also consumed low amounts of food. It is likely that, in this case, large differences in consumption can be accounted for by individual variation between birds.

Several studies have investigated songbird preferences for specific dietary antioxidants, though the results of such studies have sometimes been contradictory. Garden Warblers (*Sylvia borin*) offered a choice between a carotenoid supplemented diet and an unsupplemented diet did not show a preference for either diet (Catoni et al., 2011) and male Society Finches (*Lonchura domestica*) offered a carotenoid-enriched diet did not consume more food than birds offered a non-enriched diet (McGraw et al., 2006). It is thus possible that we did not observe a vitamin E preference in our birds because they do not select foods based on vitamin E content. However, Schaefer et al. (2008) found that Eurasian Blackcaps (*Sylvia atricapilla*) preferred diets supplemented with anthocyanins over unsupplemented food and this same species was shown to prefer food with flavonoids over food without these antioxidants (Catoni et al., 2008b). In addition, Senar et al. (2010) artificially enriched mealworms with carotenoids and found that Great Tits (*Parus major*) consistently chose enriched mealworms over non-enriched worms. More recently, Alan (2012) found that free-living birds selected wild fruits with higher levels of vitamin E over fruits with lower levels at a stopover site during autumn migration. These studies provide strong evidence for the hypothesis that, under certain conditions, birds may prefer foods supplemented with antioxidants and that they can detect these antioxidants without visual signals. During Phase 3, the two treatment groups were apparently not experiencing different levels of oxidative stress and this may have reduced the need for birds to preferentially consume diets higher in vitamin E. If the high PUFA group had sustained high levels of oxidative stress throughout Phase 3, the birds may have been unable to mount a sufficient endogenous antioxidant response to oxidative damage and this would subsequently require them to supplement their antioxidant defenses through dietary antioxidant consumption.

### 4.4. TEAC and OXY as indicators of antioxidant capacity

We compared two common measures of circulating antioxidant capacity, TEAC and OXY, in order to assess whether the two assays are directly comparable. We expected that OXY and TEAC would be positively correlated if the two assays measure similar components of antioxidant capacity. In addition, OXY was expected to correlate positively with resTEAC, but not with UA. TEAC was also expected to correlate with resTEAC. Lastly, TEAC and OXY were expected to have similar relationships with dROMs. Contrary to our expectations, OXY was not significantly related to TEAC, and resTEAC was not correlated with either OXY or TEAC (Table 3). In addition, TEAC, but not OXY, had a consistent negative correlation with dROMS (Table 3). These results confirm that the two assays measure different components of circulating antioxidant capacity and thus should not be considered directly comparable to one another. In a study comparing OXY to another common measure of antioxidant capacity, FRAP, Costantini (2011) found that the two measures did not correlate with one another, though FRAP was strongly correlated with UA. This effect, similar to the one observed in our study, may be explained by the fact that OXY uses a pro-oxidant that is endogenously produced by organisms and which can react with several kinds of antioxidants whereas FRAP simply measures the reducing ability of a compound (Costantini, 2011). As such, OXY provides a direct measure of antioxidant capacity that includes both hydrophilic and lipophilic antioxidants while FRAP only provides an indirect measure of antioxidant capacity. The same reasoning might apply when considering the lack of correlation between OXY and TEAC in our study.

There are a few practical aspects of these two assays that should be considered when deciding which measure of antioxidant capacity to use. The OXY assay is a commercial kit that is more convenient and efficient than the TEAC assay and, for us, it yielded more highly repeatable results. On the other hand, TEAC was more cost-effective and has been more widely used in avian research. Different measures of antioxidant capacity are useful for gaining an understanding of independent aspects of the antioxidant machinery and several methods may be assessed together in order to gain a more rounded understanding of antioxidant capacity (Costantini, 2011). With respect to the two assays we compared, TEAC is most useful when it is desirable to estimate the contribution of UA to circulating antioxidant capacity whereas OXY is a more desirable choice for studies in which researchers want to exclude UA from their measure of antioxidant capacity while also assessing hydrophilic and lipophilic circulating antioxidants directly.

## 5. Conclusions

To our knowledge, this study represents the first research to directly investigate the relationship between dietary protein, circulating antioxidant capacity, oxidative stress, and dietary antioxidant preference in a migratory passerine. We found that: (1) circulating uric acid increases with protein catabolism and this contributes to the antioxidant capacity of birds, (2) all birds were able to successfully mitigate the oxidative damage associated with a high PUFA diet, although the means of antioxidant defense depended on diet, and (3) none of the birds showed a significant preference for an antioxidant-rich diet. Thus, we conclude that, though uric acid may act as an important antioxidant in birds consuming a high protein diet, other antioxidant defenses are sufficiently mobilized in birds that do not have elevated levels of uric acid. In addition, these defenses are likely to be endogenous antioxidants, such as enzymatic antioxidants, that are independent of micromolecular circulating antioxidants. Though our birds did not exhibit any significant dietary preferences, likely due to the fact that they were all able to successfully mitigate oxidative stress, it is still possible that birds experiencing

sustained high levels of oxidative damage may prefer antioxidant-rich diets. Future research should investigate how oxidative stressors, particularly exercise, affect preferences for dietary antioxidants and should also quantify the response of non-circulating antioxidants (e.g. antioxidant enzymes) to oxidative damage.

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