Articles

Nutrient Profiles of Wild and Captive Attwater’s and Greater Prairie-Chicken Eggs

Michael E. Morrow,* Elizabeth A. Koutsos, John E. Toepfer

M.E. Morrow
Attwater Prairie Chicken National Wildlife Refuge, Eagle Lake, Texas 77434

E.A. Koutsos
Mazuri Exotic Animal Nutrition, PMI International, St. Louis, Missouri 61366

Present address: Koutsos Consulting, LLC, Apex, North Carolina 27502

J.E. Toepfer
Society of Tympanuchus Cupido Pinnatus, Ltd., Ada, Minnesota 56510

Present address: George Miksch Sutton Avian Research Center, Bartlesville, Oklahoma 74005

Abstract

We determined reference levels of minerals, fatty acids, and fat-soluble micronutrients in eggs from wild Attwater’s Tympanuchus cupido attwateri and two (Minnesota, Nebraska) greater prairie-chicken T. c. pinnatus populations for comparison with eggs produced by captive Attwater’s prairie-chickens to help guide formulation and evaluation of captive diets. Levels of all minerals found in wild Attwater’s prairie-chicken eggs were similar to those in at least one of the two greater prairie-chicken populations, but these levels frequently differed between the two greater prairie-chicken populations. Ratios for n-6:n-3 fatty acids were >3 times higher for Minnesota greater prairie-chickens, which had more access to waste grain than Attwater’s or Nebraska greater prairie-chickens. Captive eggs had n-6:n-3 ratios 6.7 times the pooled wild samples, while wild eggs had higher levels of anhydrolutein, zeaxanthin, β-carotene, and total carotenoids. More magnesium, zinc, and manganese were observed in wild eggs compared with those produced in captivity. Flaxseed was added to the captive breeder diet in an attempt to lower egg n-6:n-3 ratios, along with additional carotenoids found in marigold extract. These dietary modifications successfully lowered the n-6:n-3 ratio by 46%, but this ratio was still 3.6 times higher in captive eggs, consistent with the grain-based formulation of the breeder diet. Carotenoid additions successfully raised total carotenoids, but increases were primarily for lutein and not zeaxanthin or β-carotene as intended. Variability in egg nutrient composition among the three wild populations suggests that some tolerance exists in maternal diets, but impacts to offspring fitness are unknown. Given the purported importance of maternal nutrition to fitness of embryos and neonate chicks, we suggest additional research is needed to quantify the influence of key nutrient levels on offspring fitness for both captive and wild populations.

Keywords: Attwater’s prairie-chicken; captive diet; egg nutrients; greater prairie-chicken

Received: June 15, 2018; Accepted: November 29, 2018; Published Online Early: December 2018; Published: June 2019

Citation: Morrow ME, Koutsos EA, Toepfer JE. 2018. Nutrient profiles of wild and captive Attwater’s and greater prairie-chicken eggs. Journal of Fish and Wildlife Management 10(1):38–50; e1944-687X. https://doi.org/10.3996/062018-JFWM-052

Copyright: All material appearing in the Journal of Fish and Wildlife Management is in the public domain and may be reproduced or copied without permission unless specifically noted with the copyright symbol ©. Citation of the source, as given above, is requested.

The findings and conclusions in this article are those of the author(s) and do not necessarily represent the views of the U.S. Fish and Wildlife Service.

* Corresponding author: mike_morrow@fws.gov
Introduction

The Attwater’s prairie-chicken *Tympanuchus cupido attwateri* was historically abundant in coastal grasslands of southeastern Texas and southwestern Louisiana (Lehmann 1941). It was listed as endangered with extinction in 1967, and despite intensive management, fewer than 150 have existed in the wild since 1995 (US Endangered Species Act [ESA 1973, as amended]; U.S. Fish and Wildlife Service [USFWS] 2010; Morrow et al. 2015). Recovery actions have included habitat restoration, captive breeding, population supplementation and reestablishment, and intensive research to identify factors limiting wild (i.e., free-ranging) populations. All Attwater’s prairie-chickens currently in the wild were released directly from captivity or are their descendants. Substantial populations of phenotypically and ecologically similar wild greater prairie-chickens *T. c. pinnatus* still exist in the Great Plains of North America (Svedarsky et al. 2000).

A captive breeding program for the Attwater’s prairie-chicken began in 1992, and now consists of ~100 individuals held at three zoological institutions in Texas. No protocols or nutritional guidelines existed for producing Attwater’s prairie-chickens in captivity, and like most grouse (subfamily Tetraoninae), they proved difficult to rear (McEwen et al. 1969; Johnson and Boyce 1991; USFWS 2010). Mortality of ~34% has been reported for captive Attwater’s prairie-chickens during the first 10 d after hatch (USFWS 2010). Although diets for captive Attwater’s prairie-chickens were formulated using the best available science, formulations by necessity drew on information from commercial poultry literature (e.g., National Research Council 1994).

Developing bird embryos depend on nutrients deposited during egg formation to support metabolic activities necessary for growth, development, and survival. Further, the nutritional status of hens before and during laying influences not only embryo fitness, but also that of the resulting chick (Moss and Watson 1984; Wilson 1997; Papazyan et al. 2006). Such maternal effects can manifest themselves until progeny mature and even into subsequent generations (Bernardo 1996). Despite general agreement on the importance of maternal diet to offspring fitness, the precise relationships between egg nutrients and fitness are not known, even for commercially important species (Royle et al. 1999).

Considerable intra- and interspecific variation exists in reported values for egg nutrient composition (Williams 1994). Few studies have characterized egg nutrients for wild bird species; however, qualities of eggs produced by captive birds generally differ substantially from those produced by their wild counterparts (Noble et al. 1996; Speake et al. 1999). To our knowledge, no data on Attwater’s or greater prairie-chicken egg nutrient composition have been reported for either wild or captive-produced eggs. Therefore, with this study we sought to 1) determine reference values for egg nutrients and variation associated with those values for three populations of wild *T. cupido*; 2) compare egg nutrient composition of eggs produced by captive Attwater’s prairie-chickens with those reference levels to identify possible adjustments needed in the captive breeder diet; and 3) evaluate the effectiveness of breeder diet adjustments as reflected in egg nutrient composition. We evaluated three classes of nutrients: minerals (Ca, P, Mg, K, Na, Fe, Cu, Zn, Mn, and Se), fatty acids, and fat-soluble micronutrients (vitamin A, vitamin E, and the carotenoids anhydrolyeuten, lutein, zeaxanthin, and β-carotene).

Study Sites

We collected captive Attwater’s prairie-chicken eggs from the Houston Zoo, Inc. (Houston, Texas; 29.6°N, 95.1°W) and Fossil Rim Wildlife Center (Glen Rose, Texas; 32.2°N, 97.8°W). Fossil Rim and Houston are Association of Zoos and Aquariums accredited institutions, and have maintained breeding Attwater’s prairie-chickens since 1993 and 1995, respectively. Each of these facilities currently support ≥20 breeding hens/y. We collected eggs from wild Attwater’s prairie-chickens primarily from the 4,265-ha Attwater Prairie Chicken National Wildlife Refuge (Eagle Lake, Texas; 29.7°N, 96.3°W), which was established in 1972 specifically to maintain habitat for this endangered species (USFWS 2012). We also collected a few eggs (n = 3) from destroyed nests on private ranchland in Goliad County, Texas (28.6°N, 97.3°W). Both locations were within the Gulf Prairies and Marshes vegetation area of Texas (Hatch et al. 1990), and consisted of managed open grasslands dominated by native warm-season grass communities.

We salvaged wild greater prairie-chicken eggs from Minnesota (Norman and Clay counties; 47.2°N, 96.3°W) and Nebraska (Rock County; 42.4°N, 99.4°W). The Minnesota study area was dominated by fields of corn, soybeans, wheat, and sugar beets interspersed with various grassland types, scattered woodlots, and tree windrows. Privately owned grasslands were predominantly former agricultural lands enrolled in the U.S. Department of Agriculture Conservation Reserve Program, and were dominated by exotic cool-season grasses. Relatively undisturbed public grasslands (e.g., Scientific and Natural Areas [https://www.dnr.state.mn.us/snas/index.html]; Wildlife Management Areas [https://www.dnr.state.mn.us/wmas/index.html]; and Waterfowl Production Areas [https://www.fws.gov/refuges/whm/wpa.html]) containing a mix of native and exotic grasses were interspersed throughout the Minnesota study area (Toepfer 2003). The Nebraska study area was located in the expansive Nebraska Sand Hills, an area of mostly intact native tallgrass prairie interspersed with occasional agriculture (Vodehnal 1999; Chaplan et al. 2016).

Methods

We collected eggs in 2008–2014 during the April–June and May–July nesting periods for Attwater’s and greater prairie-chickens, respectively. We collected infertile eggs from captive Attwater’s prairie-chickens after initial incubation indicated the eggs were not viable. We located nests in the wild after movements of radio-
tagged hens indicated that incubation had been initiated. We chose 1 egg/clutch at random from wild Attwater’s prairie-chicken nests, whereas we collected only greater prairie-chicken eggs from destroyed nests or that were cracked in the course of monitoring but still intact. We collected only wild Attwater’s prairie-chicken eggs estimated at <7 d incubation based on telemetry data or procedures previously described (Hoyt 1979; Burnham 1983). Wild Attwater’s prairie-chicken hens were either hatched in the wild, or had been released from captivity >6 mo prior. We did not supplement diets of wild prairie-chickens except for grains available in surrounding agricultural areas.

We labeled, placed in plastic bags, and froze collected eggs until processing. We prepared samples by weighing the egg, shell, and egg contents (albumen + yolk + embryo). We staged embryos, if present, to confirm incubation was <7 d. We thoroughly homogenized egg contents using a hand-held coffee foamer. We then allocated the homogenized contents into subsamples and stored them in a −80°C freezer. We maintained egg contents under low ambient light until vitamin A, E, and carotenoid subsamples were covered in aluminum foil to avoid any light-degradation of these nutrients. We assayed samples for vitamins A, E, and carotenoids by high-performance liquid chromatography (McGraw et al. 2002; K. McGraw, School of Life Sciences, Arizona State University, personal communication). We determined complete fatty-acid profiles by gas chromatography (N-P Analytical Labs, Gratiot, Missouri). Dairy One Forage Laboratory (Ithaca, New York) determined minerals (by spectrophotometry) and dry matter composition. Diagnostic Center for Population and Animal Health, Michigan State University, assessed selenium levels only in 2013. We sent samples to respective labs for analysis within 4–5 mo of collection; we excluded samples held longer to minimize potential variation associated with storage time.

We compared data from wild eggs by collection location (Texas for Attwater’s prairie-chicken; Nebraska and Minnesota for greater prairie-chicken) for each egg nutrient. We were primarily interested in establishing reference values of central tendency and variability from wild populations for comparison with eggs produced in captivity, so we pooled samples across years. Distributions for many of the nutrient values displayed substantial deviations from normality; therefore, we used a Kruskal–Wallis rank sum test in Rcmdr 3.2-3 (Fox 2005) to compare populations. If differences (P < 0.05) were observed, we used Dunn’s test (Dunn 1964) within the dunn.test package in Program R 3.3.3 (www.r-project.org) to separate group medians.

We modified captive Attwater’s prairie-chicken breeder diets annually over 6 y (2009–2014; Table 1) after comparison of egg composition from captive and wild populations each year. Mazuri Exotic Animal Nutrition (PMI Nutrition International, St. Louis, MO) custom-formulated breeder diets offered ~2 months in advance of first expected eggs (March) through the end of the breeding season (June). We compared nutrient levels of eggs produced by captive hens with the pooled sample of wild eggs at the beginning (2008–2009 for most nutrients, 2010 for fatty acids) and the end of the study (2014) using a two-sample Wilcoxon test within Rcmdr to evaluate whether cumulative changes in the breeder diet were effective in achieving desired nutrient characteristics of eggs.

Results

We determined mineral profiles and dry matter composition for 55 wild Attwater’s prairie-chicken, 21 wild Nebraska greater prairie-chicken, 42 wild Minnesota greater prairie-chicken, and 64 captive Attwater’s prairie-chicken eggs collected during 2008–2014 (Tables S1, S2, Supplemental Material). We did not determine dry matter composition in 2009 or 2011 in order to allocate limited...
samples for other analyses. For those years, we estimated dry matter at 30% to scale other mineral parameters. We excluded from comparisons greater prairie-chicken eggs collected in 2010 because we did not conduct lab analyses on those eggs until 12 mo after Attwater’s prairie-chicken eggs. We conducted selenium analyses only in 2013 on 6 wild Attwater’s prairie-chicken, 5 Nebraska greater prairie-chicken, and 16 captive Attwater’s prairie-chicken eggs. We determined fatty acid profiles from 54 wild eggs (n = 30, 16, and 8 Attwater’s, Nebraska greater, and Minnesota greater prairie-chicken, respectively; Table S3, Supplemental Material) and 10 captive Attwater’s prairie-chicken eggs (Table S4, Supplemental Material) from 2010 to 2014. We assessed vitamins A and E, and carotenoid levels, from 105 wild eggs (n = 43, 21, and 41 Attwater’s, Nebraska greater, and Minnesota greater prairie-chicken, respectively; Table S5, Supplemental Material) and 31 captive Attwater’s prairie-chicken eggs (Table S6, Supplemental Material) collected during 2008–2014 (excluding 2010 eggs, which we did not analyze until the following year). Data S1–S3 (Supplemental Material) provide raw data for egg minerals, fatty acids, vitamins A and E, and carotenoids by source and year. Tables S1–S6 (Supplemental Material) provide complete reference values for all nutrients compared, including ranges and values of test statistics.

Wild populations

Mineral and dry matter composition of egg contents from the three wild populations are reported in Table 2. We observed no differences (P > 0.05) among the three populations for calcium, manganese, or dry matter composition. For the remaining minerals, wild Attwater’s prairie-chicken egg contents were comparable (P > 0.05) to eggs from at least one of the greater prairie-chicken populations. Selenium concentrations, assessed only in 2013 and only for wild Attwater’s and Nebraska greater prairie-chickens, were 45% higher (P = 0.01) for the Nebraska eggs. Irrespective of statistical differences, other egg mineral levels were generally similar for the three populations except for phosphorus, which was >18% lower (P < 0.001) in eggs from Minnesota.

Egg total fat (g/100 g wet weight) did not differ (P > 0.05) among the three wild populations, with medians of 14.0 for Attwater’s and Minnesota greater prairie-chickens, and 12.8 for Nebraska greater prairie-chickens (Table 2). While monounsaturated fatty acids, polyunsaturated fatty acids, and saturated fats comprised approximately equal proportions (median 4–5%) of eggs from the three populations, Attwater’s prairie-chicken eggs contained 24% more (P < 0.001) polyunsaturated fatty acids than Nebraska eggs. Median palmitic (C16:0), stearic (C18:0), oleic (C18:1n-9C), linoleic (C18:2n-6), and linolenic (C18:3n-3) levels each comprised >5% of total fatty acids for all three populations. Attwater’s prairie-chicken eggs were similar (P > 0.05) to eggs from at least one of the greater prairie-chicken populations for all fatty acids except pentadecanoic (C15:0), margaric (C17:0), vaccenic (C18:1n-7C), linolenic, arachidonic (C18:4n-6), and total n-3 fatty acids. For each of these, higher (P < 0.05) levels were observed in Attwater’s prairie-chicken eggs than either of the greater prairie-chicken populations except for vaccenic and arachidonic acids (Table 2). However, only linolenic and total n-3 fatty acids comprised >5% of total egg fatty acids. Median linolenic acid levels were 16 and 58% lower (P < 0.001) for Nebraska and Minnesota eggs, respectively, relative to Attwater’s prairie-chicken eggs. Similarly, total n-3 fatty acids were 15 and 55% lower (P < 0.001) for the two greater prairie-chicken populations, respectively, compared with Attwater’s prairie-chicken eggs. The ratio of total n-6:n-3 fatty acids for wild Attwater’s (0.9) and Nebraska greater prairie-chickens (1.0) were similar (P > 0.05), while that for Minnesota greater prairie-chickens was three times higher (P < 0.001; Figure 1).

Wild Attwater’s prairie-chicken and Nebraska greater prairie-chicken eggs did not differ (P > 0.05) in median vitamins A or E, or in median carotenoid levels (Table 2). However, Minnesota greater prairie-chicken eggs had >21% higher (P < 0.001) levels of vitamin A than either Attwater’s or Nebraska greater prairie-chicken eggs. Minnesota greater prairie-chicken eggs also displayed 14% higher (P < 0.04) median total carotenoid levels compared with those from Attwater’s prairie-chicken.

Captive vs. wild

Assuming that values observed in the three wild populations represented acceptable biological norms, we pooled these data for comparison with captive eggs (Table 3). In deference to modification of other nutrient parameters (lipid source, carotenoids), we made few changes to the mineral composition of the diet over the course of the study except copper (increased ~70% after 2010) and manganese (increased ~60% in 2010 only; Table 1). Wild eggs contained higher (P < 0.001) median magnesium (33%) and zinc (9%), while dry matter content was 6% higher (P < 0.001) for captive eggs (Table 3). Copper content of eggs did not differ by source from 2008 to 2010, but was 33% higher (P < 0.001) for captive eggs after dietary copper levels were increased. Median manganese levels were below detectable limits in captive eggs, except for 2010 when levels in the captive breeder diet were increased to 225 mg/kg. Manganese did not differ (P > 0.1) by source for that year, but were higher (P < 0.001) in wild eggs when 140 mg/kg manganese was provided in the captive diet (Tables 1, 3).

Captive Attwater’s prairie-chicken eggs collected in 2010 before dietary modification compared with pooled data from wild populations showed differences (P < 0.05) for 18 of 25 fatty acid parameters (Table 3). While total fat in 2010 captive eggs did not differ (P > 0.05) from wild eggs, captive eggs contained 17% higher (P < 0.05) median monounsaturated fatty acids and 13% higher total saturated fatty acids. Except for docosahexaenoic (C22:6n-3) acid, 2010 captive eggs were consistently lower (P ≤ 0.02) in n-3 and higher (P < 0.005) in n-
Table 2. Macro- and trace-mineral dry matter composition, fatty acid profiles, and vitamins A, E, and carotenoid content of homogenized whole-egg contents collected from three wild prairie-chicken Tympanuchus cupido populations: Attwater’s T. c. attwateri (APC; Colorado and Goliad counties, Texas), Nebraska greater T. c. pinnatus (GPC – NE; Rock County), and Minnesota greater (GPC – MN; Norman and Clay counties), 2008–2014. See Tables S1–S3 (Supplemental Material) for sample sizes, data ranges, and test statistics.

| Mineral | APC | GPC – NE | GPC – MN | P-value |
|---------|-----|----------|----------|---------|
| Ca (%)  | 0.25 ± 0.06a | 0.24 ± 0.20a | 0.23 ± 0.04a | 0.33 |
| P (%)   | 0.06 ± 0.11a | 0.09 ± 0.09a | 0.06 ± 0.14b | <0.001 |
| Mg (%)  | 0.04 ± 0.01a | 0.04 ± 0.01b | 0.03 ± 0.02a | <0.001 |
| K (%)   | 0.53 ± 0.12a | 0.51 ± 0.06ab | 0.47 ± 0.09b | 0.01 |
| Na (%)  | 0.44 ± 0.07ab | 0.46 ± 0.05a | 0.40 ± 0.08b | <0.001 |
| Fe (ppm) | 103 ± 24ab | 99 ± 22a | 112 ± 30b | 0.01 |
| Cu (ppm) | 3 ± 0a | 4 ± 1b | 3 ± 0a | <0.001 |
| Zn (ppm) | 59 ± 12ab | 64 ± 10a | 57 ± 11b | <0.001 |
| Mn (ppm) | 2 ± 2a | 2 ± 0a | 2 ± 3a | 0.67 |
| Se (ppm) | 2.48 ± 0.36a | 3.59 ± 0.61b | — | 0.01 |
| Dry Matter (%) | 29.1 ± 1.0a | 28.7 ± 2.1a | 28.0 ± 2.1a | 0.1 |

Fatty acids (% of total)

| Fatty Acid | APC | GPC – NE | GPC – MN |
|------------|-----|----------|----------|
| Myristic (14:0) | 0.50 ± 0.30a | 0.48 ± 0.13a | 0.44 ± 0.10a | 0.26 |
| Pentadecanoic (15:0) | 0.19 ± 0.03a | 0.14 ± 0.09b | 0.08 ± 0.07b | <0.001 |
| Palmitic (16:0) | 23.2 ± 2.0a | 24.4 ± 2.4ab | 26.2 ± 2.6b | <0.001 |
| Palmitoleic (16:1n-7) | 2.49 ± 0.73a | 2.88 ± 1.21b | 2.37 ± 1.06ab | 0.03 |
| Margaric (17:0) | 0.54 ± 0.16a | 0.45 ± 0.18b | 0.42 ± 0.12b | <0.001 |
| Searic (18:0) | 9.78 ± 0.56a | 8.76 ± 0.86b | 8.70 ± 0.16ab | <0.001 |
| Vaccenic (18:1n-7C) | 1.19 ± 0.26a | 1.37 ± 0.37b | 1.38 ± 0.35b | <0.001 |
| Oleic (18:1n-9C) | 26.6 ± 3.2a | 29.5 ± 5.7b | 30.5 ± 6.1ab | <0.001 |
| Eladic (18:1n-9T) | 0.11 ± 0.03a | 0.06 ± 0.12a | 0.11 ± 0.07a | 0.62 |
| Linoleic (18:2n-6) | 14.6 ± 3.0a | 14.2 ± 3.9a | 18.0 ± 4.0b | 0.03 |
| Linolenic (18:3n-3) | 15.80 ± 3.20a | 13.30 ± 7.13b | 6.66 ± 2.56b | <0.001 |
| Eicosenoic (20:1n-11) | 0.14 ± 0.06a | 0.18 ± 0.20a | 0.11 ± 0.04a | 0.27 |
| Eicosatrienoic (20:3n-3) | 0.24 ± 0.08a | 0.24 ± 0.15a | <0.10 ± 0.10b | <0.001 |
| Homo-gamma-linolenic (20:3n-6) | 0.11 ± 0.11a | 0.11 ± 0.05a | 0.10 ± 0.02a | 0.40 |
| Arachidonic (20:4n-6) | 0.44 ± 0.16a | 0.66 ± 0.16b | 0.96 ± 0.25b | <0.001 |
| Eicosapentaenoic (20:5n-3) | 0.43 ± 0.13a | 0.37 ± 0.09a | 0.22 ± 0.08b | <0.001 |
| Docosapentaenoic (22:5n-3) | 0.29 ± 0.06a | 0.24 ± 0.08ab | 0.20 ± 0.10b | 0.01 |
| Docosahexaenoic (22:6n-3) | 1.00 ± 0.11a | 1.04 ± 0.30a | 1.00 ± 0.20a | 0.70 |
| Total fat (g/100 g) | 14.0 ± 1.6a | 12.8 ± 1.8a | 14.0 ± 1.8a | 0.99 |
| Monounsaturated fat (g/100 g) | 4.0 ± 0.5a | 4.2 ± 1.0a | 4.7 ± 0.6a | 0.18 |
| Polyunsaturated fat (g/100 g) | 4.6 ± 0.9a | 3.7 ± 1.2b | 3.7 ± 0.6ab | <0.001 |
| Total saturated fat (g/100 g) | 4.6 ± 0.4a | 4.2 ± 0.7a | 4.7 ± 0.8a | 0.18 |
| Total n-3 | 18.0 ± 3.3a | 15.3 ± 7.3b | 8.1 ± 2.6b | <0.001 |
| Total n-6 | 15.2 ± 3.3ab | 15.0 ± 4.2a | 18.9 ± 4.4b | 0.02 |

Vitamins A/E = Carotenoids

| Vitamin | APC | GPC – NE | GPC – MN |
|---------|-----|----------|----------|
| Vitamin A (IU/g) | 5.35 ± 2.61a | 4.79 ± 2.35a | 6.51 ± 2.93b | <0.001 |
| Vitamin E (IU/g) | 3.87 ± 4.54a | 2.49 ± 2.09a | 2.60 ± 2.00a | 0.58 |
| Anhydrolutein (mg/kg) | 1.56 ± 1.12a | 1.83 ± 0.66ab | 2.04 ± 1.04b | 0.01 |
| Lutein (mg/kg) | 31.11 ± 11.20a | 35.40 ± 17.30ab | 35.96 ± 19.72b | 0.04 |
| Zeaxanthin (mg/kg) | 13.99 ± 6.14a | 18.47 ± 9.07ab | 18.59 ± 6.89b | 0.02 |
| β-carotene (mg/kg) | 0.40 ± 0.47a | 0.50 ± 0.63ab | 0.67 ± 0.61b | 0.01 |
| Total carotenoids (mg/kg) | 47.00 ± 16.49a | 56.31 ± 26.19ab | 54.76 ± 26.39b | 0.02 |

* Median ± interquartile range. Different letters indicate significantly different values among populations using Dunn’s multiple-comparison test with family-wise error rate controlled using a Bonferroni adjustment. Significant P-values (P ≤ 0.05) are bolded.

** Selenium levels were assessed only in 2013, and only for APC and GPC – NE samples.

6 fatty acids, resulting in median n-6:n-3 ratios 6.7 times higher (P < 0.001) than wild eggs (Figure 2). Based on these observations, sources of fats higher in n-3 fatty acids (flaxseed, fishmeal) were added to the breeder diet in an attempt to achieve a median n-6:n-3 ratio in captive eggs more similar to the 1.0 observed in the pooled wild egg sample (Table 1). Although these additions successfully lowered this ratio, eggs produced on the final 2014 diet still had median n-6:n-3 ratios 3.6 times higher (P < 0.001; Figure 2).

Median vitamin A or E levels for captive Attwater’s prairie-chicken eggs from 2008 to 2009 did not differ (P ≥ 0.28) from the pooled wild sample (Table 3). However, median anhydrolutein, zeaxanthin, β-carotene, and total
carotenoid levels were 1.7, 2.6, 7.4, and 1.3 times higher ($P \leq 0.006$), respectively, for wild eggs. Median lutein levels did not differ ($P > 0.05$) between pooled wild and captive eggs. Beginning in 2009, we added additional carotenoids (xanthophyll, β-carotene), primarily in the form of marigold extract, to the breeder diet in an attempt to modulate differences observed between wild and captive eggs (Table 1). These additions were successful in raising the median total carotenoid level observed in eggs produced by captive hens in 2014. However, much of this increase was with median lutein levels. These levels increased 1.5 times in 2014 captive eggs compared with those from 2008 to 2009. Median lutein levels in 2014 captive eggs were 47% higher ($P = 0.001$) than wild eggs, whereas there was no difference ($P = 0.78$) for the 2008–2009 captive egg sample. While median anhydrolutein and total carotenoid levels in 2014 captive Attwater’s prairie-chicken eggs were not different from those in the wild sample, 2014 captive eggs remained 56% lower ($P < 0.001$) in zeaxanthin and 69% lower ($P < 0.001$) in β-carotene despite dietary modulations.

**Discussion**

**Wild populations**

Despite several statistical differences for egg nutritional parameters among the three wild populations, median parameters generally differed by relatively small amounts except for n-3 and n-6 fatty acids (Table 2). Eggs from the Minnesota greater prairie-chicken population had a ≥3 times higher ($P < 0.001$) median n-6:n-3 than Attwater’s or Nebraska greater prairie-chickens (Table 2). Higher n-6:n-3 for Minnesota eggs likely reflected a higher proportion of waste grain in hen diets for Minnesota greater prairie-chickens compared with those from Texas or Nebraska (J.E.T., personal observation). Prairie-chickens readily make use of agricultural grains when available (Lehmann 1941; Rumble et al. 1988; Toepfer and Eng 1988). The minimum distance from an agricultural field to a nest where eggs were collected for this study in Minnesota and Nebraska was 0.5 and 12.9 km, respectively. Greater prairie-chickens in Minnesota were regularly observed feeding on waste grain, especially soybeans, during egg-laying, whereas greater prairie-chickens in Nebraska or Attwater’s prairie-chickens (Texas) were rarely observed in agricultural fields during this period. Agricultural grains are rich in n-6 fatty acids, especially linoleic (18:2n-6), compared with leaves that are rich in linolenic (18:3n-3; Speake et al. 1999; Simopoulos 2002). Our observation of n-6:n-3 fatty acid ratios in wild Attwater’s and Nebraska greater prairie-chicken eggs of ~1:1 are consistent with reports for other wild animals, and suggests that the diet of laying hens from these populations consisted of more vegetative material than for Minnesota greater prairie-chickens (Table 2; Simopoulos 2002). Cogar (1980) indicated that droppings of Attwater’s prairie-chickens from a predominantly native prairie area in Refugio County, Texas, consisted of 97% plant fragments (leaves and flowers) during spring, which would have included the peak egg-laying period. In contrast, Rumble et al. (1988) reported 14–22% composition of waste corn in the diets of North Dakota greater prairie-chickens during prenesting and incubation periods.

Whether the differences in n-6:n-3 ratios observed for Minnesota eggs were of biological significance was beyond the scope of our study. However, prairie-chickens in our Minnesota study area averaged 2.2 fewer (distributed $t_{2-tailed} = 4.84$, $P < 0.01$) chicks/brood than in Nebraska during 2012–2016 ($x = 2.6 \pm 1.1$ SD for Minnesota, and $4.8 \pm 0.7$ SD for Nebraska), which is of particular significance because reproductive success drives annual population changes in prairie-chickens (Peterson and Silvy 1994; Wisdom and Mills 1997). Little is known about the influence of fatty acid composition of wild bird eggs with regard to fitness of resulting offspring (Royle et al. 1999). Long-chain n-3 fatty acids play important roles in development of bone, neural tissue, and immunity of newly hatched chicks (Benatti et al. 2004; Cherian 2015), leading some to express concerns about skewed n-6:n-3 ratios with regard to optimal embryo development (Noble et al. 1996; Speake et al. 1999).

Hatching through the first days of life are critical periods for chicks with regard to changes in metabolic, physiologic, and environmental stressors (Cherian 2015). Smaller brood sizes compared with records from a few decades ago have been noted for several galliform species, including ring-necked pheasants Phasianus...
Table 3. Comparison of macro- and trace-mineral dry matter composition, fatty acid profiles, and vitamins A, E, and carotenoid content of homogenized whole-egg contents collected from three wild prairie-chicken (Tympanuchus cupido) populations: Attwater’s T. c. attwateri (APC; Colorado and Goliad counties, Texas), Nebraska greater T. c. pinnatus (GPC – NE; Rock County), and Minnesota greater (GPC – MN; Norman and Clay counties), with captive APC eggs from Fossil Rim Wildlife Center and the Houston Zoo, Inc. during 2008–2014. See Tables S4–S6 (Supplemental Material) for sample sizes, data ranges and test statistics.

| Minerals<sup>b</sup> | Wild | Captive - initial | Captive - 2014 |
|----------------------|------|-------------------|----------------|
| **Ca (%)** | 0.24 ± 0.04 | 0.25 ± 0.05 | — |
| **P (%)** | 0.93 ± 0.13 | 0.94 ± 0.13 | — |
| **Mg (%)** | 0.04 ± 0.01 | 0.03 ± 0.00*** | — |
| **K (%)** | 0.50 ± 0.11 | 0.48 ± 0.10 | — |
| **Na (%)** | 0.45 ± 0.07 | 0.45 ± 0.06 | — |
| **Fe (ppm)** | 105 ± 26 | 117 ± 36 | — |
| **Cu (ppm)**<sup>c</sup> | 3 ± 0 | 4 ± 2*** | — |
| **Zn (ppm)** | 58 ± 11 | 53 ± 13*** | — |
| **Mn (ppm)**<sup>d</sup> | 2 ± 2 | 0 ± 1*** | — |
| **Se (ppm)**<sup>e</sup> | 2.79 ± 0.86 | 3.35 ± 1.03 | — |
| **Dry matter (%)** | 29.2 ± 14 | 31.0 ± 1.7*** | — |
| **Fatty acids (% of total)**<sup>f</sup> | | | |
| Myristic (14:0) | 0.47 ± 0.18 | 0.52 ± 0.08 | 0.48 ± 0.06 |
| Pentadecanoic (15:0) | 0.18 ± 0.05 | 0.12 ± 0.01 | 0.00 ± 0.00*** |
| Palmitic (16:0) | 23.6 ± 2.6 | 27.3 ± 1.2*** | 25.7 ± 1.4 |
| Palmitoleic (16:1n-7) | 2.70 ± 0.75 | 3.28 ± 0.82 | 2.92 ± 0.33 |
| Margaric (17:0) | 0.52 ± 0.16 | 0.24 ± 0.09** | 0.24 ± 0.03*** |
| Searic (18:0) | 9.20 ± 1.08 | 8.93 ± 0.82 | 8.06 ± 1.08*** |
| VACCenic (18:1n-7C) | 1.26 ± 0.30 | 1.58 ± 0.15† | 1.59 ± 0.09† |
| Oleic (18:1n-9C) | 27.30 ± 4.58 | 30.05 ± 1.95 | 34.80 ± 1.78*** |
| Elaidic (18:1n-9T) | 0.11 ± 0.13 | 0.13 ± 0.04 | 0.15 ± 0.02† |
| Linoleic (18:2n-6) | 14.65 ± 3.18 | 21.60 ± 2.60** | 17.80 ± 2.22*** |
| Linolenic (18:3n-3) | 13.85 ± 5.68 | 1.47 ± 0.32*** | 3.28 ± 1.36*** |
| Eicosenoic (20:1n-11) | 0.14 ± 0.08 | 0.20 ± 0.03* | 0.23 ± 0.05** |
| Eicosatrienoic (20:3n-3) | 0.24 ± 0.14 | 0.00 ± 0.00** | 0.00 ± 0.00** |
| Homo-gamma-linolenic (20:3n-6) | 0.11 ± 0.12 | 0.16 ± 0.03 | 0.14 ± 0.01 |
| Arachidonic (20:4n-6) | 0.57 ± 0.24 | 0.97 ± 0.13*** | 0.93 ± 0.22** |
| Eicosapentaenoic (20:5n-3) | 0.40 ± 0.16 | 0.21 ± 0.06** | 0.16 ± 0.06*** |
| Docosapentaenoic (22:5n-3) | 0.26 ± 0.08 | 0.17 ± 0.09* | 0.16 ± 0.08** |
| Docosahexaenoic (22:6n-3) | 1.00 ± 0.20 | 1.44 ± 0.06*** | 1.26 ± 0.19*** |
| Total fat (g/100g) | 13.5 ± 1.9 | 14.1 ± 0.9 | 15.2 ± 1.6*** |
| Monounsaturated fat (g/100g) | 4.3 ± 0.8 | 4.8 ± 0.3 | 5.6 ± 0.5*** |
| Polyunsaturated fat (g/100g) | 4.1 ± 1.2 | 3.4 ± 0.6 | 3.6 ± 0.7 |
| Total saturated fat (g/100g) | 4.6 ± 0.7 | 5.2 ± 0.4 | 5.1 ± 0.5 |
| Total n-3 | 15.6 ± 6.2 | 3.3 ± 0.3*** | 4.8 ± 1.1*** |
| Total n-6 | 15.6 ± 3.4 | 23.1 ± 2.3*** | 19.1 ± 2.3*** |
| **Vitamins A/E + carotenoids<sup>g</sup>** | | | |
| Vitamin A (IU/g) | 5.70 ± 3.16 | 4.07 ± 3.84 | 4.83 ± 1.11 |
| Vitamin E (IU/g) | 2.60 ± 2.56 | 2.28 ± 5.13 | 4.80 ± 1.78*** |
| Anhydrolutein (mg/kg) | 1.87 ± 1.07 | 1.11 ± 0.71** | 1.23 ± 1.25 |
| Lutein (mg/kg) | 33.40 ± 12.90 | 33.74 ± 18.50 | 49.11 ± 24.00*** |
| Zeaxanthin (mg/kg) | 17.00 ± 7.69 | 6.64 ± 3.68*** | 7.50 ± 4.08*** |
| β-carotene (mg/kg) | 0.52 ± 0.65 | 0.07 ± 0.13*** | 0.16 ± 0.12*** |
| Total carotenoids (mg/kg) | 53.44 ± 18.00 | 41.51 ± 22.18*** | 58.88 ± 28.26 |

<sup>a</sup> Median ± interquartile range. Results of Wilcoxon (2-tailed) comparison of wild vs. each captive group indicated by: <em>P</em> ≤ 0.05, **0.01, ***0.001.

<sup>b</sup> Dietary mineral levels were generally unchanged during the study except as indicated for Cu and Mn. Therefore, Captive - initial values for minerals represent data collected during the entire 2008–2014 study.

<sup>c</sup> Captive dietary Cu increased from 13–14 to 21–23 ppm beginning in 2011. Captive eggs contained 3 ± 1 ppm from 2008–2010, and were not different (P = 0.61) from wild eggs.

<sup>d</sup> Captive dietary Mn was increased from 140 to 225 mg/kg in 2010 only. Captive eggs contained 1 ≥ 0 ppm, and were not different (P = 0.12) from wild eggs during that year.

<sup>e</sup> Selenium levels were assessed only in 2013.

<sup>f</sup> Captive - initial values were determined in 2008–2009.

<sup>g</sup> Captive - initial values were determined in 2010.
colchicus (Warner et al. 1999) and several populations of greater prairie-chickens (Toepfer 2003). Moss and Watson (1984) demonstrated that egg quality as influenced by maternal nutrition was one of the determinants of brood size for red grouse Lagopus lagopus and rock ptarmigan Lagopus muta.

Tremendous changes have occurred in U.S. agricultural practices over the past several decades, with potential impacts to granivorous birds. An exponential increase in the acreage of soybeans planted in the United States is of particular note (Specht et al. 2017). Broiler chicks fed raw soybeans display depressed growth due to the presence of antinutritional factors including protease inhibitors, hemagglutinins, and allergens present in raw soybeans (Coates et al. 1970; Rocha et al. 2014). Studies have suggested that raw soybeans may be problematic for wild birds such as northern bobwhites Colinus virginianus, mallards Anas platyrhynchos, and ring-necked pheasants (Robel and Arruda 1986; Loesch and Kaminski 1989; Bogenschutz et al. 1995). Our study sheds no definitive light on the relative contribution of maternal nutrition, or the consumption of raw soybeans to observed changes in brood sizes for prairie-chickens and other species; however, it suggests further research in this area is warranted.

Captive vs. wild

Despite statistical differences among several parameters upon initial comparison of nutrient characteristics from wild and captive prairie-chickens (Table 3, Figure 2), we chose to focus breeder diet refinements on n-6:n-3 fatty acids and carotenoids because of their importance to the developing embryo and neonate chick (Surai et al. 2001a,b; Speake et al. 1999; Cherian 2015). However, in view of their importance to tissue antioxidant function, our data suggest that increasing levels or bioavailability of magnesium, zinc, and manganese in the captive breeder diet may be warranted (Table 3; Papazyan et al. 2006).

The median n-6:n-3 fatty acid ratio from 2010 captive eggs was 6.7 times that of the pooled wild sample (Table 3). The largest differences were observed in higher linoleic (−47%; $P = 0.004$) and much lower linolenic (−89%; $P = 0.001$) levels in 2010 captive birds. Similar observations have been made for other captive and free-ranging and/or wild birds, and are consistent with the grain-based nature of many captive breeder diets (Noble et al. 1996; Speake et al. 1999; Cherian 2015). Fatty acids were modulated by adding flaxseed and fishmeal to the Attwater’s prairie-chicken breeder diet (Table 1). Comparison of eggs collected from captive hens in 2014 showed a 46% reduction in the n-6:n-3 as intended, but ratios in captive eggs were still 3.6 times those of wild eggs.

Both linoleic and linolenic acids are essential fatty acids, and are precursors of other long-chain n-6 and n-3 fatty acids (Neuringer et al. 1988; Cherian 2007). Both of these fatty acids are required in the diet, but their ratio is also an important consideration because of metabolic competition and different functions (Neuringer et al. 1988; Connor et al. 1992). Cherian (2007, 2015) suggested that higher n-3 fatty acids in the egg results in positive health benefits at various stages posthatch, regardless of posthatch diets in commercial poultry. Long-chain n-3 fatty acids are important to immunity of chicks and modulation of inflammatory responses (Cherian 2007, 2015). These fatty acids, especially 22:6n-3, are important for embryonic and postnatal brain and retinal development, and deficiencies in mammals have been associated with impaired learning and vision (Connor et al. 1992; Surai et al. 1999). Speake et al. (1999, 2002) suggested that relative deficiencies of n-3 fatty acids could impair neurological development of bird embryos. Survival of captive-reared birds such as the Attwater’s prairie-chicken released into the wild as part of conservation management actions is generally poor compared with wild birds (Roseberry et al. 1987; Parish and Sotherton 2007; USFWS 2010). Although many complex physical, environmental, and behavioral experiences in the captive setting undoubtedly contribute to lack of fitness upon liberation into the wild, compromised vision and learning ability would be highly detrimental to survival.

Carotenoids play an important role in an organism’s antioxidant system and immune function, along with vitamins A and E (Surai et al. 2001b; McGraw and Ardia 2004; Cherian 2015). Others have also observed substan-
tially higher total carotenoid concentrations in wild birds and free-ranging poultry compared with those in confinement (Speake et al. 1999; Papazyan et al. 2006). We successfully increased carotenoid levels of captive Attwater’s prairie-chicken eggs through the addition of marigold extract, resulting in total carotenoid levels similar to those of wild-collected eggs (Table 3). However, distribution of individual egg carotenoids was skewed toward an increase of lutein in 2014 captive eggs rather than zeaxanthin and β-carotene as intended. An inhibitory effect of lutein on β-carotene absorption has been reported (Surai et al. 2001a). Corn and eggs provide relatively high levels of dietary lutein and zeaxanthin, while green leafy materials are dietary sources of β-carotene (Perry et al. 2009). Wild hen diets consist predominantly of plant leaves and flowers found in native prairie (Cogar 1980), which undoubtedly serve as the source of these carotenoids in their eggs. Even where waste grains are available during egg laying, wild prairie-chickens still consume significant quantities of flowers and plant leaves. For example, in addition to waste corn, Rumble et al. (1988) reported dandelion Taraxacum officinale flowers, alfalfa Medicago spp., sweet clover Melilotus spp., fringed sage Artemisia frigida, and arthropods as dominant food items of North Dakota prairie-chickens during prenesting and incubation.

Conclusions/Management Implications

Despite its potential importance to embryogenesis and subsequent chick fitness, relatively little information is available on avian egg nutrient levels except for commercially important species (Williams 1996; Wilson 1997; Cherian 2015). Of the relatively few studies that have investigated egg nutrient composition of wild bird eggs, most only provide data on coarse nutrient parameters (e.g., water content, dry matter, fat content), and fail far short of the information necessary to formulate species-specific diets that will supply captive birds with nutrients commonly obtained by wild birds during egg formation. As a result, diets of captive birds, some crucial to species recovery programs like the Attwater’s prairie-chicken, are formulated based on the best information available from commercial poultry. Yet, few studies examine the adequacy of such diets. Ours is the first study that we are aware of to provide detailed data on nutrient composition of prairie-chicken eggs from either wild or captive environments.

Our observation of differences in egg nutrient values among the prairie-chicken populations we studied (wild greater prairie-chickens from Nebraska and Minnesota, wild and captive Attwater’s prairie-chickens from Texas) was not surprising. Nutrients deposited in eggs reflect maternal diets (Wilson 1997). Collection locations for wild eggs in our study spanned >2,200 km, and represented habitats ranging from relatively intact tallgrass prairie (Attwater Prairie Chicken National Wildlife Refuge, Nebraska) to a grassland matrix interspersed with agriculture (Minnesota). The differences we observed in egg nutrient composition, especially among the three wild populations that we studied, suggest that prairie-chicken embryos and neonatal chicks are tolerant to variation within maternal diets. The limits to this tolerance are unknown and likely complex. Eggs produced by wild Attwater’s prairie-chicken, many of which had been released from captivity a few months prior, generally displayed nutrient compositions within the range of variation observed for wild greater prairie-chicken populations with no history of captivity.

Ultimately, evaluation of maternal dietary composition should be conducted with regard to fitness of resulting embryos and chicks, both in the wild and in the captive environment. In particular, given the rapid expansion of U.S. soybeans, which are known to contain antinutritional factors in their raw form (Coates et al. 1970; Rocha et al. 2014), and their ready use by granivorous bird species including prairie-chickens, we suggest that research is needed to further elucidate how waste soybeans remaining in agricultural fields affect egg quality and fitness of resulting offspring. We recommend further refinement of the diet provided breeding Attwater’s prairie-chickens in captivity, especially with respect to trace minerals (magnesium, manganese, zinc), n-6:n-3 fatty acids, and carotenoids (lutein, zeaxanthin, β-carotene).

Supplemental Material

Please note: The Journal of Fish and Wildlife Management is not responsible for the content or functionality of any supplemental material. Queries should be directed to the corresponding author for the article.

Data S1. Macro- and trace-mineral dry matter composition of homogenized whole-egg contents collected from wild prairie-chicken Tympanuchus cupido populations: Attwater’s T. c. attwateri (APC; Colorado and Goliad counties, Texas), Nebraska greater T. c. pinnatus (GPC – NE; Rock County), and Minnesota greater (GPC – MN; Norman and Clay counties), and captive APC eggs from Fossil Rim Wildlife Center and the Houston Zoo, Inc. during 2008–2014. Dry matter, Ca, P, Mg, K, and Na expressed as %; Fe, Cu, Zn, Mn, and Se expressed as ppm.

Found at DOI: https://doi.org/10.3996/062018-JFWM-052.S1 (454 KB PDF).

Data S2. Fatty acid profiles (% of total fat) of homogenized whole-egg contents collected during 2010–2014 from three wild prairie-chicken Tympanuchus cupido populations: Attwater’s T. c. attwateri (Colorado and Goliad counties, Texas), Nebraska greater T. c. pinnatus (Rock County), and Minnesota greater (Norman and Clay counties) and captive Attwater’s eggs from Fossil Rim Wildlife Center and the Houston Zoo, Inc.

Found at DOI: https://doi.org/10.3996/062018-JFWM-052.S2 (341 KB PDF).

Data S3. Vitamins A and E (IU/g), and carotenoids (anhydrolutein, lutein, zeaxanthin, β-carotene, and total; mg/kg) of homogenized whole-egg contents collected during 2008–2014 from wild prairie-chicken Tympanu-
Tympanuchus cupido populations: Attwater’s T. c. attwateri (Colorado and Goliad counties, Texas), Nebraska greater T. c. pinnatus (Rock County), and Minnesota greater (Norman and Clay counties) and captive Attwater’s eggs from Fossil Rim Wildlife Center and the Houston Zoo, Inc.

Found at DOI: [https://doi.org/10.3996/062018-JFWM-052.S5](https://doi.org/10.3996/062018-JFWM-052.S5) (329 KB PDF).

**Table S1.** Macro- and trace-mineral dry matter composition of homogenized whole-egg contents collected from three wild prairie-chicken populations: Attwater’s *Tympanuchus cupido attwateri* (APC; Colorado and Goliad counties, Texas), Nebraska greater *T. c. pinnatus* (GPC – NE; Rock County), and Minnesota greater (GPC – MN; Norman and Clay counties) and captive APC eggs from Fossil Rim Wildlife Center and the Houston Zoo, Inc. during 2008–2014. Data are from Table 2 supplemented with ranges and test statistics.

Found at DOI: [https://doi.org/10.3996/062018-JFWM-052.S4](https://doi.org/10.3996/062018-JFWM-052.S4) (104 KB PDF).

**Table S2.** Comparison of macro- and trace-mineral dry matter composition of homogenized whole-egg contents collected from wild prairie-chicken populations: Attwater’s *Tympanuchus cupido attwateri* (APC; Colorado and Goliad counties, Texas), Nebraska greater *T. c. pinnatus* (GPC – NE; Rock County), and Minnesota greater (GPC – MN; Norman and Clay counties), with captive APC eggs from Fossil Rim Wildlife Center and the Houston Zoo, Inc. during 2008–2014. Data are from Table 3 supplemented with sample sizes, ranges, and test statistics.

Found at DOI: [https://doi.org/10.3996/062018-JFWM-052.S5](https://doi.org/10.3996/062018-JFWM-052.S5) (219 KB PDF).

**Table S3.** Fatty acid profiles (% of total) of homogenized whole eggs collected from three wild prairie-chicken populations: Attwater’s *Tympanuchus cupido attwateri* (APC; Colorado and Goliad counties, Texas; n = 30), Nebraska greater *T. c. pinnatus* (GPC – NE; Rock County; n = 16), and Minnesota greater (GPC – MN; Norman and Clay counties; n = 8), 2010–2014. Data are from Table 2 supplemented with ranges and test statistics.

Found at DOI: [https://doi.org/10.3996/062018-JFWM-052.S6](https://doi.org/10.3996/062018-JFWM-052.S6) (104 KB PDF).

**Table S4.** Fatty acid profiles (% of total) of homogenized whole eggs collected from three wild prairie-chicken populations: Attwater’s *Tympanuchus cupido attwateri* (Colorado and Goliad counties, Texas; n = 30), Nebraska greater *T. c. pinnatus* (Rock County; n = 16), and Minnesota greater (Norman and Clay counties; n = 8) during 2010–2014, compared with captive Attwater’s prairie-chicken eggs from Fossil Rim Wildlife Center and the Houston Zoo, Inc. during 2010 (n = 4) and 2014 (n = 6) to assess cumulative changes in captive breeder diet. Data are from Table 3 supplemented with ranges, test statistics, and P-values.

Found at DOI: [https://doi.org/10.3996/062018-JFWM-052.S7](https://doi.org/10.3996/062018-JFWM-052.S7) (235 KB PDF).

**Table S5.** Vitamins A, E, and carotenoid content of homogenized whole eggs collected from three wild prairie-chicken populations: Attwater’s *Tympanuchus cupido attwateri* (APC; Colorado and Goliad counties, Texas; n = 43), Nebraska greater *T. c. pinnatus* (GPC – NE; Rock County; n = 21), and Minnesota greater (GPC – MN; Norman and Clay counties; n = 41), 2008–2014. Data are from Table 2 supplemented with ranges and test statistics.

Found at DOI: [https://doi.org/10.3996/062018-JFWM-052.S8](https://doi.org/10.3996/062018-JFWM-052.S8) (241 KB PDF).

**Table S6.** Comparison of vitamins A and E, and carotenoids of homogenized whole-egg contents collected from three wild prairie-chicken (n = 105) populations: Attwater’s *Tympanuchus cupido attwateri* (Colorado and Goliad counties, Texas), Nebraska greater *T. c. pinnatus* (Rock County), and Minnesota greater (Norman and Clay counties) during 2008–2014, compared with captive Attwater’s prairie-chicken eggs from Fossil Rim Wildlife Center and the Houston Zoo, Inc. during 2008–2009 (n = 21) and 2014 (n = 10) to assess cumulative changes in captive breeder diet. Data are from Table 3 supplemented with ranges, test statistics, and P-values.

Found at DOI: [https://doi.org/10.3996/062018-JFWM-052.S9](https://doi.org/10.3996/062018-JFWM-052.S9) (187 KB PDF).

**Reference S1.** Lehmann VW 1941. Attwater’s prairie chicken, its life history and management. U.S. Fish and Wildlife Service, North American Fauna Series 57. Washington, D.C.: U.S. Government Printing Office.

Found at DOI: [https://doi.org/10.3996/062018-JFWM-052.S10](https://doi.org/10.3996/062018-JFWM-052.S10) (16.48 MB PDF); also available at [http://www.fws.gov/treesearch/pubs/nafa/57](http://www.fws.gov/treesearch/pubs/nafa/57).

**Reference S2.** Rumble MA, Newell JA, Toepfer JE. 1988. Diets of greater prairie chickens on the Sheyenne National Grasslands. Pages 49–54 in Bjugstad AJ, technical coordinator. Prairie chickens on the Sheyenne National Grasslands. Fort Collins, Colorado, USA.

Found at DOI: [https://doi.org/10.3996/062018-JFWM-052.S11](https://doi.org/10.3996/062018-JFWM-052.S11) (83 KB PDF); also available at [https://www.fs.usda.gov/treesearch/pubs/23842](https://www.fs.usda.gov/treesearch/pubs/23842).

**Reference S3.** Toepfer JE, Eng RL. 1988. Winter ecology of the greater prairie chicken on the Sheyenne National Grasslands. Pages 32–48 in Bjugstad AJ, technical coordinator. Prairie chickens on the Sheyenne National Grasslands. U.S. Department of Agriculture General Technical Report RM-159, Fort Collins, Colorado, USA.

Found at DOI: [https://doi.org/10.3996/062018-JFWM-052.S12](https://doi.org/10.3996/062018-JFWM-052.S12) (4.34 MB PDF); also available at [https://www.fs.usda.gov/treesearch/pubs/23842](https://www.fs.usda.gov/treesearch/pubs/23842).

**Reference S4.** [USFWS] U.S. Fish and Wildlife Service. 2010. Attwater’s prairie-chicken recovery plan. 2nd edition.
revision. Albuquerque, New Mexico: U.S. Fish and Wildlife Service.

Found at DOI: https://doi.org/10.3996/062018-JFWM-052.513 (2.86 MB PDF); also available at https://ecos.fws.gov/ecp0/profile/speciesProfile?slid=7259.

Reference S5. [USFWS] U.S. Fish and Wildlife Service. 2012. Attwater Prairie Chicken National Wildlife Refuge comprehensive conservation plan and environmental assessment. Albuquerque, New Mexico: U.S. Fish and Wildlife Service.

Found at DOI: https://doi.org/10.3996/062018-JFWM-052.514 (55.17 MB PDF); also available at https://www.fws.gov/southwest/refuges/plan/docs/Texas/APC_Final_WildlifeService.

Acknowledgments

We thank H. Bailey and M. Coym, Houston Zoo, Inc., and J. Johnson, Fossil Rim Wildlife Center, for collection of captive eggs for this study. We also thank staff and interns at the Attwater Prairie Chicken National Wildlife Refuge for assistance with egg collection and sample preparation. The Society of Tympanuchus Cupido Pinnatus, Ltd., the Minnesota Prairie Chicken Society, and the U.S. Fish and Wildlife Service provided logistical and financial support. Funders had no influence on the content of the manuscript, and did not require approval prior to publication. We thank S. Sherrod for elevating the importance of nutrition to captive breeding, and landowners for access to their land. We appreciate comments provided by two anonymous reviewers and the Associate Editor that improved an earlier version of this manuscript. This research was conducted in compliance with Guidelines to the Use of Wild Birds in Research (https://birdnet.org/info-forornithologists/guidelines-to-the-use-of-wild-birds-inresearch/guidelines-english-3rd-edition-2010/) and was authorized by the following permits: U.S. Fish and Wildlife Service TE051839, Texas Parks and Wildlife Department Scientific Research SPR-0491-384, Minnesota Department of Natural Resources 14196-17794, and Nebraska Game and Parks Scientific and Educational 234. The findings and conclusions in this paper are those of the authors and do not necessarily represent the views of the U.S. Fish and Wildlife Service or Mazuri/PMI International.

Any use of trade, firm, or product names is for descriptive purposes only and does not imply endorsement by the U.S. Government.

References

Benatti P, Peluso G, Nicolai R, Calvani M. 2004. Polysaturated fatty acids: biochemical, nutritional and epigenetic properties. Journal of the American College of Nutrition 23:281–302.

Bernardo J. 1996. Maternal effects in animal ecology. American Zoologist 36:83–105.

Bogenschutz TR, Hubbard DE, Leif AP. 1995. Corn and sorghum as a winter food source for ring-necked pheasants. Journal of Wildlife Management 59:776–784.

Burnham W. 1983. Artificial incubation of falcon eggs. Journal of Wildlife Management 47:158–168.

Chaplain S, Simms P, Dinerstein E, Carney K, Schneider R, Cook T. 2016. Nebraska sand hills mixed grasslands. Available: http://www.worldwildlife.org/ecoregions/na0809. Archived by WebCite: http://www.webcitation.org/737Dtk598.

Cherian G. 2007. Metabolic and cardiovascular diseases in poultry: role of dietary lipids. Poultry Science 86:1012–1016.

Cherian G. 2015. Nutrition and metabolism in poultry: role of lipids in early diet. Journal of Animal Science and Biotechnology 6:28. https://doi.org/10.1186/s40104-015-0029-9

Coates ME, Hewitt D, Golub P. 1970. A comparison of the effects of raw and heated soya-bean meal in diets for germ-free and conventional chicks. British Journal of Nutrition 24:213–225.

Cogar VF. 1980. Food habits of Attwater’s prairie chicken in Refugio County, Texas. Doctoral dissertation. College Station: Texas A&M University.

Connor WE, Neuringer M, Reisbick S. 1992. Essential fatty acids: the importance of n-3 fatty acids in the retina and brain. Nutrition Reviews 50:21–29.

Dunn OJ. 1964. Multiple comparisons using rank sums. Technometrics 6:241–252.

Fox J. 2005. The R Commander: a basic statistics graphical user interface to R. Journal of Statistical Software 14:1–42.

Hatch SL, Gandhi KN, Brown LE. 1990. Checklist of the vascular plants of Texas. MP-1655, College Station: Texas Agricultural Experiment Station, Texas A&M University.

Hoyt DF. 1979. Practical methods of estimating volume and fresh egg weight of bird eggs. The Auk 96:73–77.

Johnson GD, Boyce MS. 1991. Survival, growth, and reproduction of captive-reared sage grouse. Wildlife Society Bulletin 19:88–93.

Lehmann VW. 1941. Attwater’s prairie chicken, its life history and management. U. S. Fish and Wildlife Service, North American Fauna Series 57. Washington, D.C.: U.S. Government Printing Office (see Supplemental Material, Reference S1).

Loesch CR, Kaminski RM. 1989. Winter body-weight patterns of female mallards fed agricultural seeds. Journal of Wildlife Management 53:1081–1087.

McEwen LC, Knapp DB, Hillard EA. 1969. Propagation of prairie grouse in captivity. Journal of Wildlife Management 33:276–283.

McGraw K, Ardia DR. 2004. Immune-regulatory activity of different dietary carotenoids in male zebra finches. Chemoecology 14:25–29.

McGraw KJ, Adkins-Regan E, Parker RS. 2002. Anhydro-lutein in the zebra finch: a new, metabolically derived...
carotenoid in birds. Comparative Biochemistry and Physiology Part B Biochemistry and Molecular Biology 132:811–818.

Morrow ME, Chester RE, Lehnen SE, Drees BM, Toepfer JE. 2015. Indirect effects of red imported fire ants on Attwater’s prairie-chicken brood survival. Journal of Wildlife Management 79:896–906.

Moss R, Watson A. 1984. Maternal nutrition, egg quality and breeding success of Scottish Ptarmigan Lagopus mutus. Ibis 126:212–220.

Neuringer MG, Anderson J, Connor WE. 1988. The essentiality of n-3 fatty acids for the development and function of the retina and brain. Annual Review of Nutrition 8:517–541.

National Research Council. 1994. Nutrient requirements of poultry. 9th revised edition. Washington, D.C.: National Academy Press.

Noble RC, Speake BK, McCartney R, Foggin CM, Deeming DC. 1996. Yolk lipids and their fatty acids in the wild and captive ostrich Struthio camelus. Comparative Biochemistry and Physiology 113B:753–756.

Papazyan TT, Lyons MP, Mezes M, Surai P. 2006. Selenium in poultry nutrition—effects on fertility and hatchability. Praxis Veterinaria 54:85–102.

Parish DMB, Sotherton NW. 2007. The fate of released captive-reared grey partridges Perdix perdix: implications for reintroduction programmes. Wildlife Biology 13:140–149.

Perry A, Rasmussen H, Johnson EJ. 2009. Xanthophyll (lutein, zeaxanthin) content in fruits, vegetables and corn and egg products. Journal of Food Composition and Analysis 22:9–15.

Peterson MJ, Silvy NJ. 1994. Spring precipitation and fluctuations in Attwater’s prairie-chicken numbers: hypotheses revisited. Journal of Wildlife Management 58:222–229.

Robel RJ, Arruda SM. 1986. Energetics and weight changes of northern bobwhites fed 6 different foods. Journal of Wildlife Management 50:236–238.

Rocha C, Durau JF, Barrili LNE, Dahlke F, Maiorka P, Maiorka A. 2014. The effect of raw and roasted soybeans on intestinal health, diet digestibility, and pancreas weight of broilers. Journal of Applied Poultry Research 23:71–79.

Roseberry JL, Ellsworth DL, Klimstra WD. 1987. Comparative post-release behavior and survival of wild, semi-wild, and game farm bobwhites. Wildlife Society Bulletin 15:449–455.

Royle NJ, Surai P, McCartney R, Speake B. 1999. Parental investment and yolk lipid composition in gulls. Functional Ecology 13:298–306.

Rumble MA, Newell JA, Toepfer JE. 1988. Diets of greater prairie chickens on the Sheyenne National Grasslands. Pages 49–54 in Bjugstad AJ, technical coordinator. Prairie chickens on the Sheyenne National Grasslands. U.S. Department of Agriculture General Technical Report RM-159, Fort Collins, Colorado, USA (see Supplemental Material, Reference S2).

Simopoulos AP. 2002. The importance of the ratio of omega-6/omega-3 essential fatty acids. Biomedicine & Pharmacotherapy 56:365–379.

Speake BK, Surai PF, Bortolotti GR. 2002. Fatty acid profiles of yolk lipids of five species of wild ducks (Anatidae) differing in dietary preference. Journal of Zoology 257:533–538.

Speake BK, Surai PF, Noble RC, Beer JV, Wood NAR. 1999. Differences in egg lipid and antioxidant composition between wild and captive pheasants and geese. Comparative Biochemistry and Physiology Part B 124:101–107.

Specht J, Grassini P, Hoegemeyer T, Elmore R, Rees J, Mueller N, Glewen K. 2017. Soybean and corn yield and acreage trends through 2016. University of Nebraska-Lincoln. Available: https://cropwatch.unl.edu/2017/soybean-and-corn-yield-and-acreage-trends-through-2016. Archived by WebCite: http://www.webcitation.org/7365dXfQV.

Surai PF, Speake BK, Noble RC, Mezes M. 1999. Species-specific differences in the fatty acid profiles of the lipids of the yolk and of the liver of the chick. Journal of the Science of Food and Agriculture 79:733–736.

Surai PF, Speake BK, Sparks NHC. 2001a. Carotenoids in avian nutrition and embryonic development. 1. Absorption, availability and levels in plasma and egg yolk. Journal of Poultry Science 38:1–27.

Surai PF, Speake BK, Sparks NHC. 2001b. Carotenoids in avian nutrition and embryonic development. 2. Antioxidant properties and discrimination in embryonic tissues. Journal of Poultry Science 38:117–145.

Svedarsky WD, Westemeier RL, Robel RJ, Gough S, Toepfer JE. 2000. Status and management of the greater prairie-chicken Tympanuchus cupido pinnatus in North America. Wildlife Biology 6:277–284.

Toepfer JE. 2003. Prairie chickens & grasslands: 2000 and beyond. Report to the Council of Chiefs, Society of Tympanuchus Cupido Pinnatus, Ltd., Elm Grove, Wisconsin, USA.

Toepfer JE, Eng RL. 1988. Winter ecology of the greater prairie chicken on the Sheyenne National Grasslands. Pages 32–48 in Bjugstad AJ, technical coordinator. Prairie chickens on the Sheyenne National Grasslands. U.S. Department of Agriculture General Technical Report RM-159, Fort Collins, Colorado, USA (see Supplemental Material, Reference S3).

[ESA] U.S. Endangered Species Act of 1973, as amended, Pub. L. No. 93-205, 87 Stat. 884 (Dec. 28, 1973). Available: http://www.fws.gov/endangered/esalibrary/pdf/ESAall.pdf. Archived by WebCite: http://www.webcitation.org/75hIoWuYb.

[USFWS] U.S. Fish and Wildlife Service. 2010. Attwater’s prairie-chicken recovery plan. 2nd revision. Albuquerque, New Mexico: U.S. Fish and Wildlife Service (see Supplemental Material, Reference S4).

[USFWS] U.S. Fish and Wildlife Service. 2012. Attwater Prairie Chicken National Wildlife Refuge comprehensive conservation plan and environmental assessment.
Albuquerque, New Mexico: U.S. Fish and Wildlife Service (see Supplemental Material, Reference S5).
Vodehnal WL. 1999. Status and management of the greater prairie chicken in Nebraska. Pages 81–98 in Svedarsky WD, Hier RH, Silvy NJ, editors. The greater prairie chicken: a national look. Saint Paul: University of Minnesota Agricultural Experiment Station Miscellaneous Publication 99-1999.
Warner RE, Mankin PC, David LM, Etter SL. 1999. Declining survival of ring-necked pheasant chicks in Illinois during the late 1990s. Journal of Wildlife Management 63:705–710.
Williams TD. 1994. Intraspecific variation in egg size and egg composition in birds: effects on offspring fitness. Biological Reviews 68:35–59.
Williams TD. 1996. Variation in reproductive effort in female zebra finches Taenopygia guttata in relation to nutrient-specific dietary supplements during egg laying. Physiological Zoology 69:1255–1275.
Wilson H. 1997. Effects of maternal nutrition on hatchability. Poultry Science 76:134–143.
Wisdom MJ, Mills LS. 1997. Sensitivity analysis to guide population recovery: prairie-chickens as an example. Journal of Wildlife Management 61:302–312.