EFFECT OF NUTRITION ON THE REPRODUCTIVE FITNESS OF THE ENDANGERED ATTWATER'S PRAIRIE CHICKEN

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Abstract

Attwater's Prairie Chickens (*Tympanuchus cupido attwateri*) are an endangered Texan grouse species. Their survival relies on captive breeding for propagation of the free-ranging population. In recent years, chick hatchability and survivability in captivity has steadily decreased, with survivability in 2004 less than 30%. Preliminary data obtained in 2004 from captive and free ranging Attwater's Prairie Chickens, demonstrated potential differences in serum vitamin A and E concentrations. It was proposed that low hatchability and survivability could be a result of an insufficient and/or excessive supply of vitamins A and E during egg development. The goals of the studies were to; 1. Examine blood serum values from both the captive and free-ranging population for vitamin A and E; 2. Determine whether a 25% reduction in vitamin A acetate and a 42% increase in vitamin E acetate in the breeder diet of the captive birds would affect circulatory vitamin A and E concentrations 4-weeks post feeding; and 3. Determine whether these dietary formulation changes have had a significant effect on the chick hatchability and survivability at Fossil Rim Wildlife Center. Twenty eight (14.14) captive Attwater's Prairie Chickens were fed one of two breeder diets for fifteen weeks. Prior to the start and 4-weeks into the study, blood serum was collected. Serum α-tocopherol was significantly higher in the free-ranging population compared to all of the captive birds. There were no significant differences in any of the retinol values, among any of the captive or free ranging birds. Chick hatchability and survivability was higher in 2005 on both diets compared to 2004. Although it appears hatchability and survivability were affected by the diet, it is not possible to determine if improvements in reproductive success were directly linked to dietary vitamin A and/or E due to confounding factors.

Introduction

The Attwater's Prairie Chicken (APC) is an endangered North American bird species whose survival relies on captive breeding of chicks for release into the free-ranging population. Although 15 wild hens are known to have had clutches this year in the wild, no chicks have survived to adulthood. The breeding program was established in 1992 when the US Fish & Wildlife Service (USFWS) determined that captive breeding was critically important for the species. The captive breeding population was started using eggs gathered from nests at two breeding sites in southern Texas. These eggs were hatched artificially and are the founders of the captive population. As of the start of the 2005 breeding season, there were approximately 200 adult birds housed in six zoological institutions in Texas. The largest of these six facilities was Fossil Rim Wildlife Center (FRWC) in Glen Rose, Texas, with approximately 90 birds.

During the breeding season of 2004 at FRWC, egg hatchability and chick survivability was significantly lower than in previous years. Egg hatchability dropped from an average of 74.5% to 51%, and chick survivability dropped from 54% to 29%. Early, middle and late embryonic death resulted in the loss of 115 potential hatches, which was the highest death-in-shell total ever observed at FRWC. Although nutritional deficiencies were highlighted as possible causes, (metabolic bone disease, suspected vitamin E and selenium deficiencies, and vitamin B deficiencies in the chicks), other husbandry issues (viral, enteritis, over-crowding and cage substrate) could have played significant roles in these lower than average numbers.

Of particular concern to the USFWS recovery team were the concentrations of vitamins A and E in the diets of the adults and whether this was a significant cause of the poor reproductive success. In a small comparative study between APC at a free-ranging wildlife reserve and a captive facility, blood serum was analyzed for α-tocopherol and retinol.[1] Results indicated greater concentrations of circulatory α-tocopherol in the free-ranging birds compared to the captive birds (13.38 ± 4.04μg/ml; 5.79 ± 2.01μg/ml). Additionally, serum retinol concentrations were slightly less in the free ranging birds compared to the captive birds (1.19 ± 0.32μg/ml; 1.62 ± 0.28μg/ml).

Vitamin E

Vitamin E is a fat soluble vitamin that functions as an antioxidant to maintain cell membrane integrity. It is found most abundantly as α-tocopherol, although there are other isomers found naturally.[5] It is necessary for good health and successful reproduction in birds. A dietary dependant vitamin, the maternal diet is the only source of vitamin E to the developing chick, via the yolk.[10] During avian embryonic development, vitamin E protects the tissues from oxidation as a result of lipid breakdown and the actions of free radicals. Oxidation stress is greatest in the last week of development and first week post-hatch, which coincides with the greatest vitamin E concentrations in the yolk sac membrane and embryonic liver[9]. Signs of vitamin E deficiencies include low fertility, low hatchability and clinical disorders such as encephalomalacia.[3] Embryos deficient in vitamin E will often die in the final week of development or in the first week post-hatch. Other research has shown as egg clutch size increased, vitamin E concentrations within the egg yolk declined significantly.[4] These results suggested that eggs laid at the end of the clutch, from birds on marginally sufficient vitamin E diets, would have lower hatchability and higher late embryonic death.

Vitamin A

Vitamin A is an important component for the function of vision, reproduction, development, growth and health. Retinol and retinyl esters are the predominant circulatory forms of vitamin A. Deficiency in vitamin A leads to early embryonic deaths (usually within 24 hours) although absolute vitamin A deficiencies are rare.[5] Conversely, chicks from hens on marginally adequate vitamin A diets may express physiological signs soon after hatch. Signs may include anorexia, poor growth, ruffled feathers, and/or others. Unfortunately, many of the symptoms of deficiencies are somewhat similar to the signs of toxicities.[6] Chronic toxicity typically results from intakes 100 to 1,000 times nutritional requirements for prolonged periods.[7] Otherwise, normal vitamin A metabolism provides protection from toxicity. This is achieved by the conversion to the more stable, less toxic ester forms (often retinyl palmitate) which facilitates storage in the liver.[6]

Serum

Normal ranges for vitamins A and E may assist with the diagnosis of dietary adequacy for reproductive success. When determining normal ranges, serum, plasma, eggs and a number of animal tissues may be used. Either serum or plasma can be very useful, fairly non-invasive, methods of determining normal ranges, although dietary and environmental factors need to be taken into account when interpreting the results.[2,12,] Blood levels may be a reflection of intake, not necessarily of stores.[2,12]

The goals of this study were:

1. To investigate plasma concentrations of vitamin A and E in free ranging and captive APC, in the month preceding the onset of lay.
2. To determine whether a 25% reduction in vitamin A acetate and a 42% increase in vitamin E in the breeder diet of the captive birds would affect circulatory vitamin A and E concentrations 4-weeks post feeding.
3. To determine whether these dietary formulation changes have had a significant affect on the chick hatchability and survivability at FRWC.

Methods

Diet Trial

In December 2004, 14.14 adult Attwater's Prairie Chickens, consuming Mazuri™ Gamebird Maintenance Diet, were bled at FRWC. Two weeks later these birds were paired (n=14) and randomly allocated to one of two trial diet groups. The control diet (n=7) was Mazuri™ Gamebird Breeder (MGB) Regular Diet, which was also the diet fed in 2004. The test diet (n=7) was formulated by altering nutrient concentrations in the MGB Regular Diet, to formulate a slightly different ration. In addition to vitamin A and E, the test diet was higher in B vitamins, vitamin D and added xanthophylls (Table 1). The two diets were fed for a total of fifteen weeks with intakes and remaining food measured and recorded weekly. All food was measured using a standard volume cup. Cup-volume to gram-weight ratio was determined using a multiple sampling/weighing technique. Samples of the diet were collected and dried to determine dry matter (DM). Individual intakes were estimated using the mean intake for the pair. Results are presented as mean ± SD. One way ANOVA were used to determine statistical significance. In the second week of February a second serum sample was taken. In an attempt to minimize possible temperature stress all samples were taken in the early morning. All samples were stored at -20ºC for a short period of time prior to transportation to the Fort Worth Zoo (FWZ) nutrition department where they were stored at -80ºC until analyzed.

Free Ranging Birds

Serum samples were collected from 7.13 APC opportunistically caught at the APC National Wildlife Reserve on the evenings of the 14th – 16th February 2005. All blood samples were collected using the same protocol assigned for the FRWC birds. Serum was kept chilled, then frozen to -20ºC on return to the facility, and shipped to FWZ on dry ice where they were stored at -80ºC until analyzed.

Vitamin Analysis

Vitamins (α- and γ-tocopherol, retinol and retinyl esters) were extracted using HPLC methodologies[8]. All results are presented as means ± SD. One-way ANOVAs and Tukey's comparisons were used to determine statistical significance.

Results and Discussion

The change in diet had no statistically significant effect on overall consumption across the experimental design, with pre-lay intake the only value coming close to significance ($P=0.059$) (Table 2). Observational data suggested an increase in consumption of the MGB Test diet was seen in the first couple of weeks. One of the speculations was the higher consumption was due to a small "novelty" effect. The new pellet was a slightly different shape, smell and color, which could have caused this increased intake.

Though no statistical differences were observed in total dietary dry matter consumed, the MGB Test diet contained a higher concentration of vitamin E thus vitamin E intake was increased (Table 2). It was hypothesized with the increased intake in vitamin E, circulatory serum α-tocopherol also would increase. However there was no significant difference observed between the two diets. It was speculated the increased α-tocopherol may be stored and/or partitioned for egg development. Analysis of the egg yolks would verify this theory. There were significant differences in the free-ranging α- and γ-tocopherol values compared to those in captivity. Free ranging serum α-tocopherol values were almost double the captive values. Captive and free-ranging values exceed domestic chicken 'normals' and values from birds with suppressed α-tocopherol due to excessive vitamin A supplementation.[11] Additionally these values were at the low-end or within serum ranges seen in other captive galliformes.[3] Higher levels in the free-ranging birds could be attributed to diet, but free ranging dietary ingredients have yet to be quantified. The γ-tocopherol levels were significantly higher in all of the captive birds, most likely due to the corn and soybean oil, two of the base ingredients of the captive diet.

There were no differences in the circulatory retinol among the MGB Maintenance, MGB Test, MGB Regular or the free ranging population (Table 3). Values were higher than published data for domestic chickens[11]. Retinyl palmitate was highest in the birds on the MGB Regular Diet and was significantly higher than the concentrations found in the free ranging population (Table 3). However, no differences were observed between the birds on the maintenance and/or on the MGB test diet for retinyl palmitate (Table 3). The physiological significance of this is difficult to determine. In order to better interpret these increased retinyl palmitate values, additional data are needed including: liver values, additional serum values and published values on laying hens. No signs of vitamin A toxicity were observed in any pathology, nor were visible signs noted in living birds. MGB test diet had additional carotenoids added but there was no increase in circulatory retinol. It was subjectively observed that birds on the MGB Test Diet were significantly brighter in color than those on the regular breeder diet.

In 2005, it appeared the eggs from hens consuming the MGB Regular Diet had the highest fertility, the lowest embryonic mortality, and the highest hatchability, when compared to eggs produced from hens consuming either the MGB Test Diet or those on the MGB Regular in 2004. Intake of the MGB Regular in 2004 was not quantified. Fertility and embryonic death comparisons with the free-ranging birds cannot be determined, since these data were not collected, but hatchability compared to all eggs laid, was greater in the free ranging environment than the captive birds. Additionally, chicks hatched from adults consuming the MGB Regular Diet had the greatest survivability to 2-weeks compared to all other treatments including the free-ranging populations. Notably, survivability in the captive institution was higher than the survivability in the free-ranging population. Although there is evidence to suggest that hatchability and survivability can be attributed to the diet, it is not possible to determine if improvements in reproductive success from 2004 to 2005 was directly linked to dietary vitamin A and/or E. There are numerous factors that effect embryonic mortality (egg storage prior to incubation, incubator and/or the incubator conditions, etc), 'healthy' hatches (genetics, incubation, etc) and chick survival to 2-weeks (diet, disease, stocking density, etc).

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TABLES

Table 1: Calculated nutritional analysis of the Mazuri™ Gamebird breeder regular, Mazuri™ Gamebird breeder test and Mazuri™ Gamebird maintenance diets (dry matter basis).

Nutrients	MGB Breeder Regular Diet	MGB Breeder Test Diet	MGB Maintenance
Protein, %	22.00	22.00	13.86
Fat, %	4.40	4.40	3.30
Linoleic Acid, %	2.20	2.20	1.65
Fiber (Crude), %	4.62	4.62	4.73
Metabolizable Energy, kcal/g	2.79	2.79	3.10
Ash, %	10.01	10.01	4.84
Calcium, %	3.04	3.04	0.88
Phosphorus, %	0.63	0.63	0.34
Potassium, %	0.96	0.96	0.62
Magnesium, %	0.35	0.35	0.22
Sodium, %	0.21	0.21	0.17
Chloride, %	0.35	0.35	0.31
Sulfur, %	0.29	0.29	0.19
Iron, ppm	297.00	297.00	121.00
Zinc, ppm	143.00	143.00	100.10
Manganese, ppm	143.00	143.00	110.00
Copper, ppm	15.40	15.40	10.12
Iodine, ppm	1.76	1.76	1.43
Selenium, ppm	0.55	0.55	0.45
Cobalt, ppm	0.08	0.08	0.15
Vitamin K, ppm	0.70	0.70	0.84
Vitamin A, IU/kg	**21,450.00**	**15,950.00**	6,600.00
Vitamin D3(added), IU/kg	2,585.00	2,585.00	2,475.00
Vitamin E, IU/kg	**189.20**	**330.00**	121.00
Carotene, ppm	2.75	2.75	0.48
Thiamin Hydrochloride, ppm	**9.79**	**19.58**	11.00
Riboflavin, ppm	**12.10**	**22.00**	5.50
Niacin, ppm	**121.00**	**165.00**	105.60
Pantothenic Acid, ppm	**23.10**	**33.00**	11.00
Choline Chloride, ppm	1622.50	1622.50	1100.00
Folic Acid, ppm	3.63	3.63	3.30
Pyridoxine, ppm	**11.00**	**22.00**	6.82
Biotin, ppm	0.29	0.29	0.44
Vitamin B12, mcg/kg	24.20	24.20	16.50
Xanthophylls, ppm	**8.80**	**96.80**	0

Table 2: Mean dry matter (DM), vitamin E and vitamin A consumption of Mazuri™ Gamebird breeder (MGB) test and regular diets for 15 weeks pre-, during- and post-lay.

	MGB Test Diet			MGB Regular Diet		
	Pre-lay	During-lay	Post lay	Pre-lay	During-lay	Post-lay
DM consumed (Est. g/day)	46.48 ± 4.918^{a}	47.50 ± 4.562^{a}	39.85 ± 5.296^{a}	42.75 ± 3.1838^{a}	47.97 ± 5.608^{a}	38.56 ± 8.063^{a}
Vit E calc. cons. (Est. IU/day)	13.94	14.25	11.96	7.353	8.251	6.632
Vit A calc. cons. (Est. IU/day)	673.96	688.75	577.83	833.62	935.41	751.92

DM intake presented as means ± SD. Values not sharing a common superscript differ significantly (P<0.05)

Table 3: Mean serum α-tocopherol, γ-tocopherol, retinol and retinyl palmitate values from captive Attwater's Prairie Chickens consuming Mazuri™ Gamebird maintenance diet; Mazuri™ Gamebird breeder test, and Mazuri™ Gamebird breeder regular diet; and from free-ranging Attwater's Prairie Chickens at the National Wildlife Reserve, Eagle Lake, Texas.

	Maintenance	Mazuri Breeder Diets		Free Ranging (2005)
		Test	Regular	
α-tocopherol	5.49 ± 1.477^{a}	5.68 ± 1.910^{a}	6.35 ± 1.697^{a}	12.62 ± 1.956^{b}
Γ-tocopherol	0.34 ± 0.963^{a}	0.42 ± 0.131^{a}	0.35 ± 0.135^{a}	0.15 ± 0.074^{b}
Retinol	2.28 ± 0.437^{a}	2.19 ± 0.690^{a}	2.34 ± 0.564^{a}	2.24 ± 0.284^{a}
Retinyl palmitate	0.091 ± 0.042^{ab}	0.085 ± 0.031^{ab}	0.121 ± 0.058^{a}	0.076 ± 0.024^{b}

All values in µg/ml; Presented as means ± SD. Values not sharing a common superscript differ significantly (P<0.05)

Table 4: Total production numbers from adult hens producing chicks in 2005 and 2004 at Fossil Rim Wildlife Center and the Free Ranging population.

	Mazuri™ Gamebird Breeder Diets			Free Ranging#
	Test	GB Breeder		
	2005	2005	2004	2005
Total No of Females	7	7	36	13
Total Females producing eggs	7	5	31	13
No of Eggs	97	106	457	168
Fertile (% Fertility)	74 (76%)	95 (83%)	350 (77%)	---
Embryonic deaths (Early, Middle, Late)	25 (34%) (2, 10, 13)	17 (18%) (3, 5, 9)	115 (33%) (46, 16, 53)	---
Hatched	49 (66%)	78 (82%)	235 (67%)	146 (86%)
Wryneck*	4	20	26	0
Hatched Healthy	45	58	209	146
Survivability (to 2 weeks)	29	43	62	83
% Survivability	64.44%	74.14%	29.67%	56.85%

*Wryneck is a condition that is speculated to be phenotypically linked. All chicks born with wryneck are euthanized at hatch and are thus removed from calculations.

#Data collected by Mike Morrow, Refuge Manager APC NWR, Eagle Lake, Texas