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Hyperlipidemia and reproductive failure in captive-reared alligators: vitamin E, vitamin A, plasma lipids, fatty acids, and steroid hormones

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Abstract

Blood samples were collected from 26 captive-reared alligators (25 females; one male) and 12 (seven females and five males) wild 'nuisance' alligators collected by wildlife personnel in south Louisiana in May 1995. The captive alligators, hatched from artificially incubated eggs in 1972–1973, had received vitamin E supplements during the 3 weeks before the blood sample was collected. Each sample was analyzed for vitamin E (α-tocopherol), vitamin A (retinol), total lipid, triacylglycerol, phospholipid, cholesterol, cholesteryl ester, free fatty acids, steroid hormones and a standard clinical blood panel. The fatty acid composition of the plasma lipid fraction was also analyzed. Results indicated that 18 of the captive females and three of the seven wild females were undergoing vitellogenesis, i.e. had elevated plasma estradiol and elevated plasma calcium. Vitellogenic females had higher vitamin E than non-vitellogenic females (77.4 μg/ml vs. 28.6 μg/ml in captive females; 24.0 μg/ml vs. 21 μg/ml in wild females). Plasma retinol was similar in all groups, ranging from 0.5 to 1.4 μg/ml and close to values reported in birds. All lipid fractions, with the exception of cholesteryl ester, were higher in captive alligators than in wild alligators. There were also significant differences in the fatty acid composition of wild and captive alligators. Plasma eicosapentaenoic and docasahexaenoic acid were higher in wild than in captive alligators, whereas linoleic was higher in captive than in wild.

Keywords: Alligator; Plasma lipids; α-Tocopherol; Retinol; Hormones; Reproduction; Hyperlipidemia; Fatty acids

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1. Introduction

Alligators and crocodiles maintained in captivity are generally fed diets high in saturated fats. As a result of this overly rich diet, steatitis (Frye, 1973; Larsen et al., 1983; Wallach and Hoessle, 1968), vitamin E deficiency (Dierenfeld, 1989) and gross obesity are common in captive crocodilians. Reproduction in captive animals is also commonly impaired. High concentrations of saturated fats in the diet have been shown to result in vitamin E deficiency and reproductive failure in a wide variety of vertebrates. The vitamin E deficiency is believed to be caused by lipid peroxidation of the dietary fat, especially if the diets are frozen and thawed (Scott, 1978). Vitamin E deficiency is a known cause of reproductive failure and early embryonic death in mammals and birds (Dolensek et al., 1979; Jensen, 1968; Scott, 1978), and is a likely cause of similar pathologies in reptiles. Supplementing vitamin E to the diet has been reported to improve captive breeding in the Indian mugger crocodile, *Crocodylus palustris* (Singh and Sagar, 1991), and the gharial, *Gavialis gangeticus* (Singh, 1989). In a previous study of captive alligators, we reported that wild female alligators had higher circulating concentrations of vitamin E than captive-reared female alligators, but trace elements and hormone levels were not significantly different between the two groups (Lance et al., 1983). Although captive alligators have been reported to reproduce, the eggs they do produce are generally inferior to those collected from wild alligators (Elsey et al., 1994). When the eggs from both groups are artificially incubated under identical conditions, eggs from wild alligators show an approximately 90% hatch rate, whereas hatching rates in eggs from the captive group rarely exceed 50% and are often much lower (Elsey et al., 1994; Lance et al., 1983). Noble et al. (1993) hypothesized that the poor hatching performance in eggs from captive alligators could be due to a lack of particular fatty acids in the diet, because eggs and embryos of farm–reared alligators were low in C20 and C22 polyunsaturated fatty acids as compared to eggs and hatchlings of wild alligators. Captive Nile crocodiles, however, also had significantly lower levels of C20 and C22 plasma fatty acids than wild crocodiles (Mopurgo et al., 1993), but were reported to have reproduced in captivity (Mopurgo, personal communication). Early attempts at improving egg quality by supplementing the diet of this group of captive alligators with omega-3 fatty acids met with limited success, and no eggs were recovered from treated animals for lipid analysis (Elsey, unpublished). The alligators in this study were given large doses of vitamin E for a period of 6 weeks immediately before mating and nesting for 3 consecutive years. We present data on plasma vitamin E and vitamin A (retinol), plasma lipids and fatty acid profiles, plasma steroid hormones and minerals in these captive animals, and in wild adult alligators.

2. Materials and methods

2.1. Animals

Blood samples from 26 adult alligators maintained in captivity at the Rockefeller Wildlife Refuge in Grand Chenier, Louisiana, and blood samples from 12 'nuisance' wild alligators taken by wildlife personnel in the same area were used in this study. The age of each of the captive alligators was known since they were hatched from artificially incubated eggs in 1972–1973. Mean lengths for the captive and wild alligators were 274.4 and 248.9 cm, respectively (body mass was not recorded). Of the 26 captive alligators, only one was a male. Seven females and five males comprised the wild group.

The captive alligators were fed ground nutria (*Myocaster coypus*) meat once a week with a commercial vitamin mix added at the time of feeding. In 1993, some of the females received an additional supplement of 1200 IU vitamin E. In 1994 and 1995, the dosage was increased to 2400 IU vitamin E per week. Alligators in Louisiana do not feed during the winter months, i.e. from late October until late March or April of the following year (Joanen et al., 1981; Joanen and McNease, 1987). Vitamin E supplement was initiated when the animals first began to feed in April and discontinued 6 weeks later at the time of nesting. To ensure that each animal received the vitamin supplement, gelatin capsules were inserted into ground meat sausages and offered on the end of a pole.

In May 1995, a 20-ml blood sample was obtained from wild and captive alligators via the caudal vein using a 30-cm^3 heparinized syringe with an 18-gauge, 38-mm needle. The captive

alligators (body mass > 80 kg) were caught by a noose when they emerged to feed and a blood sample was collected. The estimated body mass of the wild alligators ranged from 30 to 50 kg. The blood was stored on ice until the plasma was separated using a clinical desk-top centrifuge. Aliquots of the plasma were immediately placed in foil-wrapped vials to prevent the oxidation of tocopherol by ultra-violet light and stored on dry ice and then in an ultracold freezer at −70°C until assayed. The remaining plasma aliquots were stored at −20°C until assayed.

2.2. Chemicals

All organic solvents used were of pesticide grade. Chloroform, methylene chloride, methanol, methyl *tert*-butylether, acetic acid, acetone, ethyl acetate and 2-propanol were from Fisher Scientific (Pittsburgh, PA). Diethyl-ether (anhydrous) and hexane (96% *n*-hexane) were from J.T. Baker (Phillipsburg, NJ). Cholesterol, cholesteryl palmitate, oleic acid, triolein, phosphatidyl choline, phosphatidyl serine, phosphatidyl inositol, phosphatidyl ethanolamine, nonadecanoic acid (C19:0), and heinecosanoic acid (C21:0) were purchased from Sigma Chemical Co. (St. Louis, MO). Dipalmitoyl phosphatidyl choline was purchased from Cal Biochem (San Diego, CA). Phospholipid standards (#1127) were purchased from Matreya Inc. (Pleasant Gap, PA). BCA Protein Assay Reagent was purchased from Pierce (Rockford, IL). Methanolic borontriflouride and fatty acid methyl ester standards were obtained from Supelco Industries (Bellefonte, PA). Compressed nitrogen (high purity grade), helium (zero grade), hydrogen (zero grade) and air (zero grade) were purchased from Air Products Inc. (Allentown, PA).

2.3. Vitamins E and A

The extraction of vitamins A and E and standard preparations were performed as described by Lee et al. (1992). Briefly, 200 μl of plasma was extracted in 400 μl of butanol/ethyl acetate (1:1). After adding 20 mg of sodium sulfate, the sample was centrifuged at $14\,000 \times g$ for 2 min. The upper organic layer was removed and stored in amber vials at −20°C until analysis. Retinol, retinyl acetate (internal standard), and α-tocopherol were purchased from Sigma (St. Louis, MO, USA). HPLC analysis was performed by isocratic elution on a Waters Model 501 with only slight modifications from Lee et al. (1992). The column used was a Supelcosil LC8 (25 × 4.6 mm). The mobile phase consisted of methanol/butanol/water (89.5:5:5.5). The flow rate was set at 1.0 ml/min and 20 μl of the organic extract was injected. Retinol and retinyl acetate were detected at 340 nm and α-tocopherol was detected at 292 nm. Retention times for retinol, retinyl acetate, and α-tocopherol were 5.1, 6.0 and 10.05 min, respectively (Fig. 1).

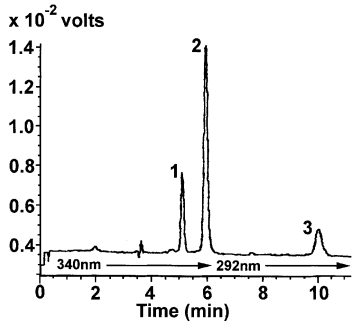

Fig. 1. Trace of an HPLC assay of alligator plasma. Peak 1: retinol; peak 2: retinyl acetate (internal standard); peak 3: α-tocopherol. Retention times and detection wavelength used are indicated on the *X*-axis.

2.4. Lipid extraction

For each individual, an aliquot of plasma (400 μl) was extracted according to the method of Bligh and Dyer (1959) for lipid analysis. Extracted lipid samples were weighed to the nearest 0.1 μg and stored in 500 μl of nitrogen-saturated methylene chloride/methanol (1:1) under a nitrogen atmosphere at −80°C until assaying.

2.5. Lipid class determination

Samples of approximately 20 μg from each lipid extract were spotted in duplicate onto activated S-III Chromarods in 1–2 ml of methylene chloride/methanol (1:1) for thin layer chromatography/flame ionization detection (TLC/FID) lipid class analysis on an Iatroscan TH-10 TLC/FID Analyzer (Iatron Laboratories, Tokyo, Japan). To control for lipid class retention time

and FID efficiency during each sample analysis, two chromarods were spotted with a standard mixture of phosphatidyl choline, cholesterol, triolein and cholesteryl oleate in proportions similar to that found in alligator plasma (15, 0.5, 8 and 4 μg, respectively). Rods spotted with lipids were refocused twice in chloroform/methanol (1:1) and then developed in hexane/diethyl ether/formic acid (85:15:0.1) for 45 min. Racks containing the chromarods were then dried at 100°C for 5 min before being scanned. Developed chromarods were scanned at 30 cm/min. Gas flow rates for hydrogen and air were 190 and 20 ml/min, respectively. Peak areas were quantified using standard curves for each lipid class component. Periodic checks of area responses for standards indicated that standard errors of replicates were in all cases less than 3% of mean values.

2.6. Saponification, methylation and GLC analysis

The plasma extract (500 mg) was utilized for GLC analysis of fatty acids. An aliquot of 25 μg each of C19:0 and C21:0 fatty acids were added to each lipid sample as internal standards for GLC analysis. Direct *trans*-methylation was accomplished by adding 3 ml of 5% methanolic HCl and 1 ml of methylene chloride to each sample, then capping each tube under a nitrogen atmosphere and incubating at 70°C for 2 h. After cooling to room temperature, 5 ml of 6% K_2CO_3 and 2 ml of methylene chloride were added to each sample. Each tube was then vortexed and the lower phase containing the fatty acid methyl esters was removed, dried under a stream of nitrogen, resuspended in 500 μl of methylene chloride and moved to a crimp top vial for GLC analysis.

Fatty acid methyl esters were analyzed on a Hewlett-Packard 5890A gas chromatography system equipped with a flame ionization detector using a J&W DBWAX fused silica capillary column (30 m × 0.25 mm i.d., J&W, Folsom, CA). The carrier gas was helium at a flow rate of 1.0 ml/min. Injector and detector temperature were 300°C and the oven temperature was programmed from an initial temperature of 50°C for 2 min to 200°C in 16 min, from 200°C to 210°C in 11 min, and from 210°C to 220°C in 18 min. Peaks were identified by comparison with retention times of known standards and by elution order as determined by GC/mass spectroscopy using an identical capillary column.

2.7. Hormones and plasma inorganic ions

Testosterone, estradiol-17β, progesterone and corticosterone were measured using radioimmunoassay as described previously (Lance, 1989; Lance and Elsey, 1999; Lance et al., 1983). Plasma calcium was analyzed by atomic absorbtion spectrophotometry. Magnesium, phosphorus, sodium, chloride, and potassium were analyzed on a Hitachi 911 auto-analyzer.

2.8. Statistics

The data were subjected to a single factor analysis of variance followed by Sheffé's multiple range test.

3. Results

The blood samples were collected during the period when females were in breeding condition and, if breeding that year, undergoing vitellogene-

Table 1
Plasma vitamin E and retinol in captive and wild alligators[a,b]

	n	Vit. E	Range	Retinol	Range
Captive breeder	18	77.4 ± 8.3	35.7–152.2	0.9 ± 0.03	0.7–1.2
Captive non-breeder	7	26.8 ± 2.8	18.9–38.5	0.8 ± 0.1	0.5–1.0
Wild breeder	3	24.0 ± 3.8	19.9–31.7	1.0 ± 0.1	0.8–1.2
Wild non-breeder	4	21.0 ± 1.4	18.5–24.2	0.8 ± 0.1	0.6–1.0
Wild male	5	18.4 ± 2.4	10.9–26.0	0.9 ± 0.2	0.5–1.4
Captive male	1	17.7		0.89	

[a] Values are in μg/ml. Each number represents the means ± S.E. of the mean for each group.
[b] CB, captive breeding females; CN, captive non-breeding females; WB, wild breeding females; WN, wild non-breeding females; WM, wild males; CM, captive male. Numbers of individuals as in Table 1. Values in mg/ml.

Table 2
Plasma lipids in captive and wild alligators[a]

	CB	CN	WB	WN	WM	CM
Cholesterol	0.61 ± 0.04	0.20 ± 0.06	0.47 ± 0.11	0.17 ± 0.02	0.17 ± 0.01	0.11
Cholesterol esters	0.31 ± 0.04	0.41 ± 0.07	0.53 ± 0.11	0.58 ± 0.06	0.49 ± 0.07	0.22
Triacylglycerol	14.83 ± 1.11	2.13 ± 1.66	9.70 ± 2.11	0.17 ± 0.06	0.09 ± 0.01	0.10
Phospholipids	4.7 ± 0.11	0.96 ± 0.37	2.17 ± 0.04	0.53 ± 0.02	0.44 ± 0.10	0.28
Free fatty acids	0.075 ± 0.014	0.049 ± 0.024	0.020 ± 0.002	0.022 ± 0.002	0.026 ± 0.002	0.05
Total lipids	20.50 ± 2.12	3.75 ± 2.05	2.88 ± 2.18	1.47 ± 0.05	1.22 ± 0.02	0.76

[a]CB, captive breeding females; CN, captive non-breeding females; WB, wild breeding females; WN, wild non-breeding females; WM, wild males; CM, captive male. Numbers of individuals as in Table 1. Values in mg/ml.

sis. Results from the breeding females, i.e. those exhibiting elevated estradiol levels, were analyzed separately from non-breeding females. Vitellogenic females have higher circulating lipids and higher phosphorus and calcium than non-breeding females (Lance et al., 1983).

A good separation and quantification of vitamin E (α-tocopherol) and vitamin A (retinol) was achieved with the HPLC system employed. A typical elution profile is shown in Fig. 1.

Concentrations of plasma vitamin E are presented in Table 1. In the captive group, a dietary supplement of vitamin E resulted in circulating levels in the breeding females that were three times higher than the levels in the breeding females from the wild. This difference, however, was not significant due to the small sample size of the wild group. No significant difference due to sex was observed for vitamin E among the wild group. Vitamin E in the captive breeding group, however, was significantly higher than the levels in captive non-breeders, wild non-breeders, and wild males ($P < 0.001$). There were no significant differences among any of the groups for plasma vitamin A (Table 1).

Plasma lipid data are presented in Table 2. In most instances, the differences between the groups failed to reach significance using the Sheffé's *F*-test. Cholesteryl esters, phospholipids and free fatty acids showed no significant differences. All lipid fractions, with the exception of cholesteryl esters, which were lower in captive breeders, were higher in captive breeding females than all other groups. Cholesterol, triacylglycerol (TAG) and total lipids all showed the same statistical differences and all were significantly higher in captive breeders than captive non-breeders, wild non-breeders, and wild males ($P < 0.001$).

Plasma lipid fractions by percent of total are given in Table 3. Wild non-breeding females showed an almost identical plasma lipid profile to that of wild males, whereas in captive female alligators, the principal plasma lipid component was triacylglycerol.

A comparison of the plasma fatty acids between male and female wild alligators, and captive female alligators is presented in Table 4. The percentage of C18:0, C20:4n6, C20:5n3 and C22:6n3 were significantly lower in the captive females than the wild females ($P < 0.05$). Linoleic acid, C18:2n6, on the other hand was significantly higher in the captive females than in the wild alligators ($P < 0.05$).

The results of the plasma steroid hormone analyses are shown in Table 5. Breeding females had higher estradiol and testosterone levels than non-breeding females ($P < 0.001$), and there was no difference between wild and captive breeders.

Table 3
Percent plasma lipids in captive and wild alligators[a]

	CB	CN	WB	WN	WM	CM
Cholesterol	2.99	5.38	3.61	11.60	13.62	14.41
Cholesterol esters	1.53	10.85	4.11	39.07	40.64	28.96
Triacylglycerol	72.34	56.88	75.28	11.73	7.38	13.36
Phospholipids	22.76	25.61	16.83	36.02	36.20	37.35
Free fatty acids	0.36	1.30	0.15	1.49	2.13	6.02

[a]Abbreviations as in Table 2.

Table 4
Plasma fatty acid composition of wild and captive alligators[a]

Fames	Wild males	Wild females	Captive females
C14:0	1.25 ± 0.29	1.84 ± 0.53	0.88 ± 0.08
C16:0	18.55 ± 1.69	19.82 ± 1.14	18.50 ± 2.85
C16:1n7	4.39 ± 0.82	5.02 ± 0.83	3.85 ± 1.09
C16:2n4	0.88 ± 0.17	0.86 ± 0.06	0.60 ± 0.16
C16:3n4	1.05 ± 0.22	1.06 ± 0.26	0.58 ± 0.19
C18:0	7.57 ± 0.55	6.75 ± 0.47	5.25 ± 0.68*
C18:1n9	11.85 ± 0.94	12.40 ± 2.47	16.44 ± 3.66
C18:1n7	2.91 ± 0.44	2.77 ± 0.13	2.35 ± 0.49
C18:2n6	7.84 ± 1.65	8.28 ± 2.91	15.06 ± 0.87*
C18:3n6	0.10 ± 0.10	0.09 ± 0.06	0.00 ± 0.00
C18:3n4	0.07 ± 0.07	0.21 ± 0.06	0.05 ± 0.03
C18:3n3	1.39 ± 0.21	2.11 ± 0.81	3.65 ± 1.12
C18:4n3	1.93 ± 0.75	1.92 ± 0.36	2.78 ± 1.44
C20:1n9	0.12 ± 0.09	0.17 ± 0.05	0.14 ± 0.04
C20:1n7	0.10 ± 0.10	0.02 ± 0.02	0.05 ± 0.02
C20:4n6	6.18 ± 1.21	4.74 ± 0.17	3.35 ± 0.57*
C20:4n3	1.33 ± 0.55	1.17 ± 0.33	2.07 ± 1.11
C20:5n3	5.83 ± 1.16	5.79 ± 1.93	0.82 ± 0.15*
C22:5n3	1.38 ± 0.30	0.95 ± 0.27	0.38 ± 0.10
C22:6n3	4.92 ± 1.51	5.21 ± 1.43	1.62 ± 0.26*

[a]Female lipid samples represent both breeding and non-breeding combined.
*Significantly different from wild females $P < 0.05$.

Plasma corticosterone and aldosterone were significantly higher in the wild ('nuisance') alligators than in the captive alligators ($P < 0.05$).

Breeding female alligators also had significantly higher plasma calcium and magnesium than non-breeding females ($P < 0.001$). None of the other measured components showed statistical differences (Table 6).

Phosphorus was generally higher in breeding females than in males and non-breeding females, but the differences were not significant, despite the close association of calcium and phosphorus (Fig. 2).

Comparison between captive and wild males could not be made because there was only one captive male sampled in the study.

4. Discussion

The first obvious difference between wild and captive alligators is in body mass. The captive alligators were all much heavier and more obese than wild alligators of similar total length. This result is not surprising as captive alligators (and zoo-maintained crocodilians in general) clearly eat more and are less active than their wild counterparts.

The alligators in this study are kept in large mixed-sex groups in semi-natural conditions (Joanen et al., 1981; Joanen and McNease, 1987).

Table 5
Plasma steroid hormones in captive and wild alligators[a]

	CB	CN	WB	WN	WM	CM
Estradiol pg/ml	327 ± 40	33 ± 11	323 ± 67	24 ± 6	–	–
Testosterone ng/ml	1.38 ± 0.17	0.2 ± 0.04	1.2 ± 0.4	0.28 ± 0.02	19.8 ± 14	57.8
Corticosterone ng/ml	2.79 ± 0.38	3.30 ± 0.5	20.7 ± 7.8	48.7 ± 20.5	9.6 ± 4.0	3.3
Aldosterone pg/ml	10.4 ± 1.2	25.2 ± 11.3	44.8 ± 20.5	209 ± 99	35.2 ± 22.1	16.8

[a]Abbreviations as in Table 2. Number of individuals as in Table 1.

Table 6
Plasma ions in captive and wild alligators[a]

	CB	CN	WB	WN	WM	CM
Protein (g/dl)	6.2 ± 0.3	5.7 ± 0.2	4.7 ± 0.6	5.0 ± 0.3	5.2 ± 0.3	5.7
Calcium (mmol/l)	7.96 ± 0.20	3.32 ± 0.18	6.28 ± 0.36	3.09 ± 0.31	3.19 ± 0.34	2.98
Phosphate (mmol/l)	3.1 ± 0.09	1.87 ± 0.16	2.35 ± 0.22	3.81 ± 1.9	2.03 ± 0.32	1.39
Magnesium (mmol/l)	1.59 ± 0.04	1.09 ± 0.04	1.44 ± 0.08	1.29 ± 0.28	0.98 ± 0.16	1.03
Sodium (mmol/l)	151 ± 1.5	149 ± 4.4	146 ± 4.3	148 ± 3.3	145 ± 3.8	150
Potassium (mmol/l)	5.2 ± 0.1	4.5 ± 0.4	4.2 ± 0.2	4.9 ± 1.6	4.50 ± 0.43	4.70
Chloride (mmol/l)	118 ± 1.2	114 ± 2.4	113 ± 3.8	109 ± 2.4	108 ± 2.4	112
Glucose (mmol/l)	4.28 ± 0.18	4.25 ± 0.25	4.75 ± 0.97	6.74 ± 2.14	6.82 ± 0.79	4.05

[a]Abbreviations as in Table 2. Number of individuals as in Table 1.

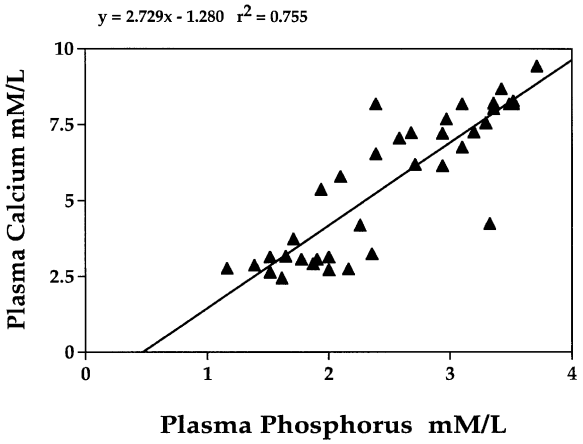

Fig. 2. A scatter plot of alligator plasma calcium and phosphorus to indicate the close relationship between the two components.

Identification of individual animals is almost impossible until they are caught and the identification tags read. Thus, it was not feasible to follow an individual alligator over several weeks of treatment. This group of captive alligators had received a vitamin mix that included vitamin E, added to their weekly feed for more than 10 years (Joanen and McNease, 1987). The vitamin mix was merely sprinkled on the ground meat and often left in the full sunlight. Under these conditions it was unlikely that many of the alligators received a substantial dose of vitamin E. The additional vitamin E supplement was an attempt to increase egg quality and percent hatching in general and not designed to monitor individuals. Not all females received the additional vitamin supplement, and it was not possible to verify which of those that did, thus a number of the captive females that failed to get the treatment had plasma vitamin E levels that were not significantly different from the wild alligators. This result would suggest that the vitamin mix that was routinely added to the feed was insufficient to elevate levels of vitamin E in captive alligators above those of wild alligators, but that the additional supplement was effective in raising circulating levels.

Vitamin E in the captive female alligators was higher than in the wild alligators. Nevertheless, the captive alligators undergoing vitellogenesis had higher vitamin E than the captive females not undergoing vitellogenesis. The sample sizes from the wild alligators were too small for statistical comparisons, but there were no obvious differences among the different groups. The elevated vitamin E in the breeding females is probably due to the lipid binding property of the yolk precursor lipoproteins which are known to bind lipid soluble dyes and incorporate them into the yolk (Astheimer et al., 1989). In a previous study, more than 10 years earlier, on these same alligators, vitamin E levels were compared to wild alligators using a different assay technique (Lance et al., 1983). The results of the two studies give remarkably similar results. A mean vitamin E level of 22 μg/ml for wild adult alligators during the month of April (Lance et al., 1983) is very close to the mean of 24 μg/ml found in this study. The samples in the 1983 study were not kept protected from light or frozen at −80°C, as in this study, and thus overall values were lower and showed much more variability, ranging from as low as 1.0 μg/ml to a high of 48.4 μg/ml. The mean value for males was less than 8 μg/ml in the earlier study, whereas in this study, the mean value in wild males was 18.4 μg/ml. Alligator vitamin E levels are similar to those reported for avian species (Calle et al., 1989; Crissey et al., 1998) and are up to 10 times higher than the levels reported for mammals (Brush and Anderson, 1986; Dierenfeld, 1989). Plasma concentrations of vitamin A levels are also higher in birds than in mammals (Schweigert et al., 1991), but there is no published data on plasma vitamin A (retinol) in reptiles. There were no differences in plasma vitamin A between wild and captive alligators or between males and females. The values we report here for the alligator are in the same range as those found in birds (Schweigert et al., 1991; Wallace et al., 1996).

Plasma lipids in the captive female alligators were extremely high. For all classes of plasma lipids, with the exception of cholesteryl esters, circulating levels in the captive females were significantly higher than in wild individuals, even when vitellogenesis is taken into account. For example, triacylglycerol values in captive breeding females ranged from 10.4 to 26.8 mg/ml, and in captive non-breeding females from 0.2 to 9.3 mg/ml. Triacylglycerol in wild alligators ranged from 0.06 to 13.3 mg/ml. The hyperlipidemia in the captive females is especially apparent when triacylglycerol levels are compared with the captive non-breeding and the wild non-breeding females. The captive non-breeding alligators had a mean plasma triacylglycerol of more than 2.0

mg/ml, whereas the mean value in the wild females was only 0.2 mg/ml. Total cholesterol and phospholipid values showed a similar dichotomy. The elevated plasma cholesterol levels in the captive alligators were less than one-third the extremely high cholesterol values reported for captive Nile crocodiles in Israel fed a diet of chicken necks, fish and day old chicks (Mopurgo and Gelman, 1991). These hyperlipidemic Nile crocodiles did, however, produce some eggs, but information on percent fertility, hatchability and number of years of successful reproduction were not available (Mopurgo, personal communication). Cholesteryl esters in the females did not show the same pattern as was seen for the other lipid fractions and were in fact higher in non-breeding females and males than they were in vitellogenic females. A similar lack of association between vitellogenesis and plasma cholesteryl esters was seen in the plasma of vitellogenic desert tortoises (Lance unpublished). The lipid composition of plasma from vitellogenic females (72% TAG, 22% PL) is almost identical to that of the alligator egg yolk (Noble et al., 1993). TAG in the non-breeding, captive female alligators, however, was still a major component of the plasma lipid whereas in wild non-breeding females, it is in the same range as wild male alligators. What was particularly striking in males and non-reproductive females was that a significant portion of the plasma lipid was cholesteryl esters, whereas in breeding (vitellogenic) females it was only a minor component of the plasma. Interestingly, plasma TAG was not higher in the single captive male than in wild male samples, and all other fractions in this individual were lower than in wild males.

The lipid composition of fat tissue from farm-reared alligators in Florida was directly related to the diet upon which they were fed. The differences were particularly notable for those alligators fed fish vs. those alligators fed beef. In one farm, fat from fish-fed alligators contained 11.1% docosahexaenoic acid and 4.0% eicopentaenoic acid, while fat from beef-fed alligators at another farm contained only trace amounts of these fatty acids (Peplow et al., 1990). The pattern of fatty acids in the plasma of captive vs. wild alligators (Table 5), also shows this pattern; C20:4n6; C20:5n3 and C22:6n3 were all significantly lower in the captive than the wild females despite there being sufficient precursors (i.e. 18:2n6 and 18:3n3, respectively), available for elongation and desaturation (Sprecher et al., 1995). This suggests that alligators have a low capacity for the synthesis of highly unsaturated fatty acids (HUFA) from 18 carbon precursors and must receive these HUFAs from the diet. In addition, if the low levels of C22:6n3 found in captive females were reflected in similar values in captive males, such a deficiency may have significant effects on sperm function. In many species, C22:6n3 is an important component of sperm tail lipids (Lin et al. 1993), and thus may also be a contributory factor in reproductive failure in captive crocodilians. Plasma fatty acids of wild and captive Nile crocodiles also display this difference in polyunsaturated fatty acids (Mopurgo et al., 1993). A similar difference was noted in the fatty acid composition of eggs and embryos of wild vs. captive-reared alligators. Lipids from the yolk of captive alligators contained much lower levels of C20 and C22 polyunsaturated fatty acids and higher levels of C18 polyunsaturated fatty acids than yolk from eggs collected from wild alligators (Noble et al., 1993). This pattern was also seen in the fat content of various tissues of the embryos from wild and captive alligators (Speake et al., 1994). Egg yolk lipids from wild and captive ostrich show a similar difference (Noble et al., 1996). Interestingly, plasma from tuatara (*Sphenodon punctatus*) in New Zealand also showed lower C20 and C22 polyunsaturated fatty acids in captive-reared than in wild tuatara (Gartland-Shaw et al., 1998). This difference in the fatty acid composition of wild and captive birds and reptiles appears to be a widespread phenomenon and may, as suggested by Noble et al. (1993), be the cause of poor reproductive performance in many captive animals.

The changes in plasma calcium, magnesium, phosphorus, protein and steroid hormones during vitellogenesis have been reported previously for alligators (Lance et al., 1983; Lance, 1987, 1989). Associated with the rise in estradiol and ovarian follicular growth are significant increases in calcium, zinc, magnesium, phosphorus, protein and lipid. In this study, 18 of the 25 captive females showed evidence of vitellogenesis, whereas only three of the wild females showed follicular development and the associated elevated plasma estradiol. The plasma hormone levels are similar to what was previously reported for the alligator with the exception of corticosterone and aldo-

sterone. These were elevated in the wild alligators as a result of stress (Lance and Elsey, 1999). These animals were held out of water for some time before a blood sample was taken, and hence corticosterone and aldosterone were elevated indicating both dehydration and stress. The stress also resulted in elevated levels of potassium, phosphate and glucose.

In 1999, necropsies were carried out on a number of the captive alligators used in this study. In a preliminary assessment of gross and histological data a large number of reproductive tract pathologies in the females were observed, none of which could be directly attributed to vitamin E deficiency or lipid peroxidation damage. The males appeared to be normal and no steatitis was seen in any of the animals (Lance, Pappendick and Elsey, in preparation). Thus, reproductive failure in this group of captive alligators is not necessarily the result of fatty acid or vitamin deficiencies alone, some other factors are also contributing to their failure to reproduce.

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