PROCEEDINGS

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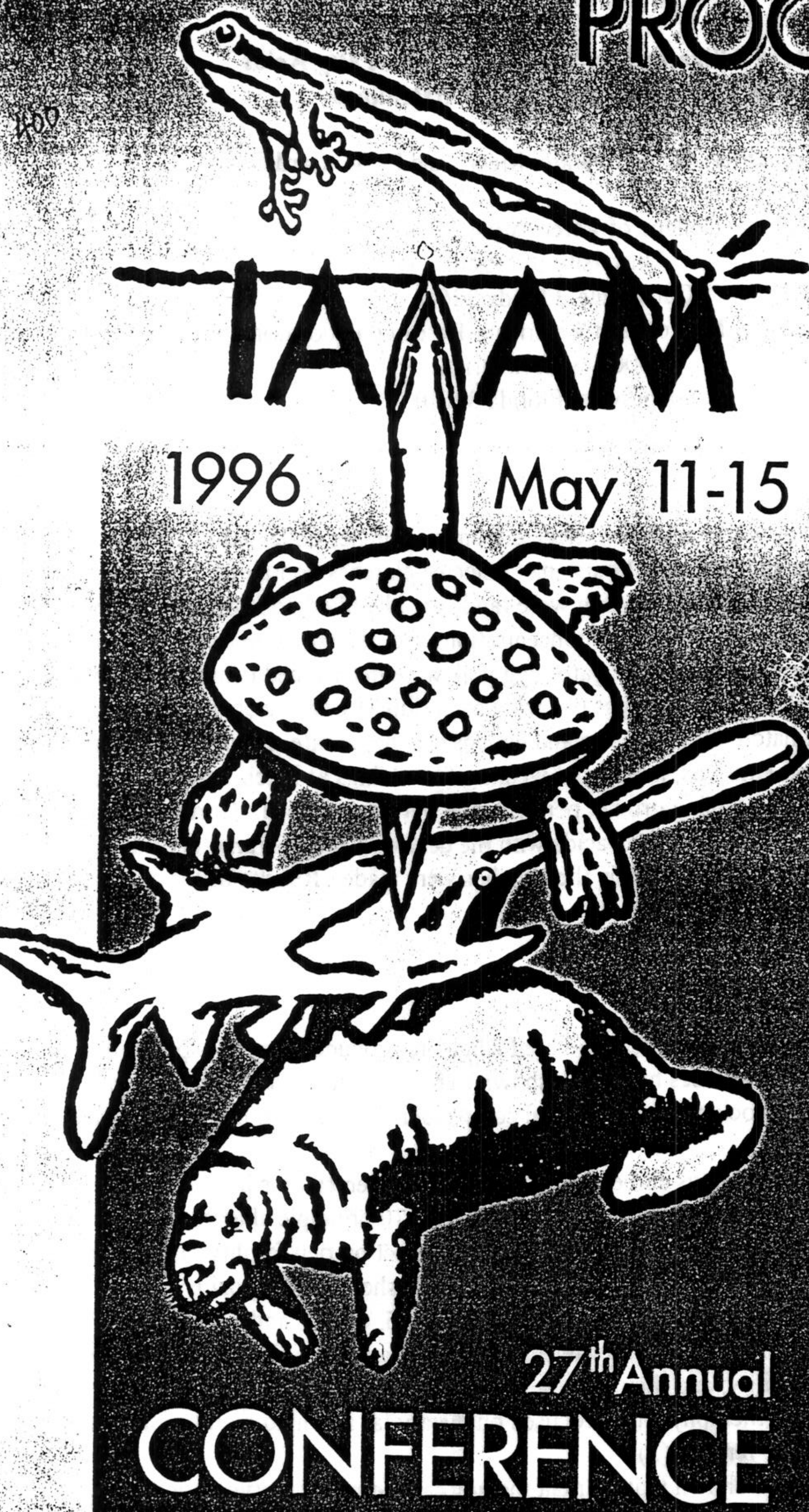

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Effects of Alligator Egg Yolk Long-Chain Fatty Acids on Growth Performance of Hatchlings

E. L. Haeussner, R. K. Vander Meer, and P. T. Cardeilhac

College of Veterinary Medicine
University of Florida
Gainesville, Florida 32610
USDA/ARS Medical and Veterinary Research Laboratory
1600 SW 23rd Drive
Gainesville, Florida 32610

ABSTRACT

Nine clutches of eggs were randomly selected from all clutches collected in 1995 at the Rockefeller Wildlife Refuge in southwest Louisiana. Selected clutches contained 3 or more infertile eggs and 12 or more fertile eggs which were randomly identified and collected as the experimental sample from each clutch. Mean concentrations and percent composition of the 10 principle long-chain fatty acids present in alligator egg yolk were determined using yolks from the 3 infertile eggs from each clutch. Fertile eggs (12 from each clutch) were incubated under optimal laboratory conditions until hatch. The egg size and dimensions, hatch rate, embryonic deaths, developmental stage of dead embryos, length and weight of hatchlings at hatch, efficiency of embryonic development, and hatchling growth performance were determined. Correlations of percent composition for each of the 10 fatty acids with the developmental parameters and growth performance of hatchlings from fertile eggs in the clutches were made. Results of the study are presented in this paper.

INTRODUCTION

Infertility and embryonic death in captive-bred alligators produce a major expense for alligator farmers in Florida (1). Losses have been shown to be associated with management practices such as pen design, stocking density, social compatibility, sex ratios, breeder age, and feed management (1).

A link between fertility of eggs and egg-yolk fatty acid concentrations have been demonstrated (3). An earlier report from this lab identified the 10 principle alligator egg yolk long-chain (AEYLC) fatty acids (Table 1) and analyzed their affect on pen fertility rates of captive breeders. Those AEYLC fatty acids whose concentrations were shown to be positively associated with fertility (C16, C16:1, C18:1. C18:3, C20:4, C20:5, C22:5, and C22:6) were designated the fertile AEYLC fatty acids. However, the concentrations of C18 and C18:2 were negatively associated with fertility; and they were designated the infertile AEYLC fatty acids. The results suggested that nutrient theraphy which focused on dietary fat and lipid metabolism could be successful in improving egg-fertility (3).

Preliminary studies have shown that nutrient therapy can increase embryo survival in fertile eggs with a resultant improvement in hatch rates (1). A reasonable assumption is that the AEYLC fatty acids may influence early hatchling development and performance. This study was performed to evaluate the affects of the relative percent concentrations of the 10 principle AEYLC fatty acids on hatchling development and performance.

Table 1. Principle Alligator Egg Yolk Long-Chain (AEYLC) Fatty Acids.

COMMON NAME	SYSTEMATIC NAME	ABBREVIATION
Palmitic acid	hexadecanoic acid	C16
Palmitoleic acid	9-hexadecanoic acid	C16:1 (n-7)
Stearic acid	octadecanoic acid	C18
Oleic acid	9-octadecanoic acid	C18:1 (n-9)
Linoleic acid	9,11-octadecanoic acid	C18:2 (n-6)
alpha-Linolenic acid	9,12,15-octadecanoic acid	C18:3 (n-3)
Arachidonic acid	5,8,11,14-eicosatetraenoic acid	C20:4 (n-6)
Timnodonic acid	5,8,11,14,17-eicosapentaenoic acid	C20:5 (n-3)
Clupanodonic acid	7,10,13,16,19-docosapentaenoic acid	C22:5 (n-3)
Cervonic acid	4,7,10,13,16,19-docosahexaenoic acid	C22:6 (n-3)

MATERIALS AND METHODS

Source of Eggs

Nine clutches of alligator eggs were randomly selected from all clutches collected in 1995 at the Rockefeller Wildlife Refuge in southwest Louisiana. An experimental sample consisting of 12 fertile and 3 infertile eggs were randomly collected from each clutch. The eggs were transported by automobile to Gainesville, Florida. Upon arrival, the fertile eggs were placed into trays sorted by clutch and incubated under optimal laboratory conditions until hatch.

Egg Morphometry

Morphometric measurements were performed with each fertile egg. Included in the measurements were egg length (l), width (wd), and weight (wegg). The calculated volume of each egg (CALCVOL) was determined by the formula: CALCVOL = 4.1888 x l x wd.

Hatchling Development and Performance

Measurements of hatchlings were taken on the day of hatch (Day 1), on Day 56, and on Day 99. Parameters included hatchling length (ln), weight (wt), body condition (cct), and performance (pf) and rate of gain in length (grt) for each measurement period. Period 1 consisted of Day 1 to Day 56. Period 2 consisted of Day 56 to Day 99. Period 3 consisted of Day 1 to Day 99. Sample formulas for body condition, performance, and rate of gain in length are given below (numbers indicate period of measurement):

cc2t = (((log wt2) / 3) x ln2 x 1000) - 300
grlt = ((ln2 - ln1) / (period 1)) x 1000
pfl = (grlt + cc2t) / 2
htcyldeg = (wt1 - wegg) x 100

Any egg which failed to hatch was opened and the stage of embryonic death was determined. Each egg morphometric measurement and hatchling developmental parameter was averaged based on the clutch of origin.

Fatty Acid Determinations

Regarding the percent concentrations of the AEYLC fatty acids, there is a high degree of correlation between fertile and infertile eggs within a clutch (3). Therefore, in an effort to spare the fertile eggs for hatchling parameter evaluation, infertile eggs were used to determine the mean percent concentrations of the AEYLC fatty acids for each clutch.

Lipids were extracted from the infertile egg yolks by the Folch method (2). The lipid polar fraction was separated by salicic acid chromatography and fatty acids associated with the polar fraction were transesterified by standard methods. Gas chromatography was used to identify the fatty acid esters by comparison with known standards. Previous studies in this lab have shown a close correlation between fatty acid concentrations in the polar and neutral fractions; but concentrations for the n-3 group were higher in the polar fraction and, consequently, changes in concentration easier to detect (3). The 10 principle AEYLC fatty acid esters were selectively pooled into a single group. The concentration of each fatty acid as a percent of the total concentration of the AEYLC fatty acid group was determined, and clutch means were calculated.

Statistics

Statistics were performed using the SAS system, OS/2 version (SAS Institute, Cary, North Carolina). Correlations between the mean percent concentrations for each of the 10 fatty acids and the mean hatchling developmental parameters for infertile eggs and fertile eggs, respectively, from the same clutch were calculated. Additionally, the individual AEYLC fatty acids were combined into a variety of secondary groups based on similar chemical structures, association with fertility, and apparent similarities in correlations with specific growth parameters. These fatty acid group names and group compositions are given in Table 2. Correlations between these groups and the growth parameters were calculated.

Table 2. Secondary Groupings of AEYLC Fatty Acids.

Group Name	Fatty Acid Components of Group
INFERTFA	C18 + C18:2
FERTFA	C16 + C16:1 + C18:1 + C18:3 + C20:4 - C20:5 + C22:5 + C22:6
OMEGA-3FA	C18:3 + C20:5 + C22:5 + C22:6
OMEGA-6FA	C18:2 + C20:4
C18FA	C18 + C18:1 + C18:2 + C18:3
C16FA	C16 + C16:1
HCHYLDFA	C16 + C20:5 + C22:5 + C22:6
LOYLDFA	C18:1 + C18:2 + C18:3 + C20:4
HIEGVLFA	C18:2 ± C18:3
LOEGVLFA	C16 + C16:1 + C20:5 + C22:5 + C22:6
EDEATHFA	C16:1 + C22:5 + C22:6
ESURVFA	C18:1 + C18:2 + C20:4

RESULTS

Results of the correlations are presented in Table 3 and Table 4. Under the heading of each growth parameter, the strength of each linear relationship was assigned a category based on whether a positive (+) or a negative (-) association exists and on the absolute value of the Pearson correlation coefficient (r) as follows:

Table Value	Pearson Coefficient	Degree of Association
1	$0.30 < \lvert r \rvert < 0.40$	suspect correlation
2	$0.40 < \lvert r \rvert < 0.50$	slight correlation
3	$0.50 < \lvert r \rvert < 0.60$	moderate correlation
4	$0.60 < \lvert r \rvert < 0.80$	strong correlation

No relationships were found to have a Pearson coefficient greater than $r = 0.80$.

Table 3. Correlation of Yolk Fatty Acid Concentrations with Embryonic Development and Hatchling Growth Parameters.

Fatty Acid	16	16:1	18	18:1	18:2	18:3	20:4	20:5	22:5	22:6
Egg Volume	-3	-2			+3	+4		-1	-3	-3
Embryonic Death		+1		-1	-1		-1		+2	+4
Stage at Death					+1		+4			-3
Hatchling Yield from Egg	+3			-3	-1	-2	-1	+2	+3	+3
Hatchling Length Day 1	-2				+2	+1	+2	-1	-3	-1
Condition Day 1	+1						-3	+2	+4	+2
Condition Day 56				+1			-1		+2	
56 Day Growth Rate							+1	-1	-3	-3
56 Day Performance				+2		-1				

Table 4. Correlation of Combinations of Yolk Fatty Acid Concentrations with Embryonic Development and Hatchling Growth Parameters.

Fatty Acid Group Abbreviation	Egg Calc. Volume	Emb. Death Rate	Emb. Stage of Death	Hatch-ling Yield from Egg	Hatch-ling Length Day 1	Hatch-ling Cond. Day 1	Hatc h-ling Cond. Day 56	56 Day Grow Rate	56 Day Perf.
INFERT	+4	-1	+2	-2	+3		-2		-1
FERT	-4	+1	-1	+2	-3	+1	+1	-1	
OMEGA-3	-2	+2		+3	-2	+3		-3	
OMEGA-6	+3	-1	+2	-1	+3				
ALL C18	+4	-2		-3					
ALL C16	-4	+1		+2	-1				
HIYLD	-3	+1		+3	-2	+2		-1	
LOYLD	+3	-2	+1	-3	+1				
HIEGGVOL	+4	-1	+1	-1	+2				
LOEGGVOL	-3	+2		+3	-1	+1		-1	
EMBDEATH	-3	+4	-2	+2	-1			-1	
EMBSURV	+3	-2	+1	-2	+1				

DISCUSSION

The results of this study indicate that the AEYLC fatty acids identified by Millstein et al (1994) influence hatchling developmental parameters in addition to their affects on fertility rate. However, except for a slight correlation with fatty acid C18:1, early hatchling performance is not significantly associated with the percent concentrations of these fatty acids.

It has been suggested that the yolk fatty acid profile reflects dietary lipid composition (1). Research on dietary therapy provides some support to this observation (3). Therefore, an analysis of yolk fatty acid profiles of captive alligator eggs may lead investigators to determine if certain of these fatty acids are present in higher or lower concentrations relative to the other AEYLC fatty acids. Results of such studies on a small sample of eggs may assist the farm manager to determine if any modification in dietary content would be beneficial.

Because eggs from wild alligators were used in this study, the affects of breeder diets on early hatchling development and performance were not experimentally analyzed. However, nutrient therapy has been demonstrated to increase fertility in captive alligators (3). Therefore, one might surmise that nutrient therapy focusing on the lipid composition of the diet might be useful for increasing certain hatchling parameters such as early hatchling length and rate of growth and for reducing embryonic death. The correlations presented in Table 3 and Table 4 might be useful in this regard.

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