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Brad Fenwick, Editor
Department of Pathology and Microbiology
College of Veterinary Medicine
Kansas State University
Manhattan, KS 66506

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# DIETARY THERAPY FOR EGG FERTILITY IN THE AMERICAN ALLIGATOR: AN EVALUATION BY DETERMINING FATTY ACID PROFILES OF EGG YOLK

S. R. Millstein[1], R. K. Vander Meer[2] E. M. Schoenagle[1] and P. T. Cardeilhac[1]

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

## INTRODUCTION

Fertility of adult females in a breeding colonies or wild populations of alligators is based on the average number of viable hatchlings produced annually by each female. Normal fertility for the prime adult female alligator is considered to be 20 and the level of fertility for a colony is the percent of 20 represented by the mean viable hatchling yield for each female in a colony (1). Colony-Infertility is defined as 100 minus the level of fertility. Infertility is a major problem in captive alligator breeding colonies with the average colony-infertility level for Florida farms about 90% (1). Management related causes of colony-infertility such as pen-design, stocking-density, social-compatibility, sex-ratios, breeder-age and feed-management have been identified and studied (1). Although most of the identified causes of infertility could be corrected, proper feed management was more difficult to accomplish because recommendations for correct practical-feed composition and feeding rates have only recently been described (1).

Infertile-eggs are a major contributor to colony-infertility and before treatments were begun on Florida farms in the early 1980's about 30% of the eggs laid were found to be infertile (1). For practical purposes, infertile eggs are defined as those which fail to form a grossly visible opaque band (associated with lack of development of the Chorioallantois).

Studies on dietary therapy showed that variations in each of five groups of nutrients: protein, total fat, highly unsaturated fat, vitamins and antibiotics, had different effects on reproductive parameters of the alligator (1,2). Total fat composition of the diet had a strong correlation ($r = 0.58$) with egg-infertility (2). It seemed plausable that the fatty acid composition of the egg yolk would reflect the dietary differences in fat consumption (1,4). In this report we describe an approach to identify and quantitate the 10 principle alligator egg yolk long chain (AEYLC) fatty acids from polar lipids. We discuss the use of the information in evaluating egg quality (egg-fertility; extent of embryonic development before death) and the effectiveness of nutrient therapy.

## MATERIALS & METHODS

### Egg-Fertility

Egg-fertility was established by fertility of the clutch that served as the source for eggs. Fertility of the clutch is the percentage of eggs in a clutch that show a grossly visible opaque band after 14 days of incubation at 31° C. Individual clutches cannot always be identified in breeding pens; therefore, it is necessary to use fertility of the pen, determined in the same manner as fertility of the clutch.

Source of Eggs

The data set consisted of a sample of 106 eggs selected from 44 clutches. Clutch fertility for all eggs ranged from 0 to 100% with a mean value of 55.8%. Clutch-size ranged from 24 to 49 with a mean value of 40.1. Pen-fertility was used for eggs in the nutrient therapy study because clutch-fertility was not known. The eggs were collected from the wild in Louisiana and Florida and from three farms in Florida. Twenty-two (22) eggs from 22 clutches came from Farm H in south Florida; 13 eggs from 13 clutches came from a pen which had been treated for infertility (pen-fertility = 86%) and 9 eggs came from 9 clutches in the untreated control pen (pen-fertility = 77%). Treated and untreated pens were selected because of their similarity in breeder-age, stocking-density, and sex-ratio (females/males).

High and Low Fertility Eggs

The data set was sorted and "high-fertility" eggs were those coming from clutches having a fertility greater than 86% and "low-fertility" eggs were those coming from clutches having a fertility less than 14%.

Egg Morphometry

The following measurements were made on each egg: length, width, weight and volume by water displacement. Percentage of egg weight represented by the shell and by the membrane were determined when possible.

Nutrient Therapy

Farm H was used to test the effects of preconceptual nutrient therapy on egg fertility. Similar treatment and control pens were selected, each with 15 year old breeders at approximately the same stocking densities (17 and 13 breeders, respectively, per acre) and sex ratios (females/males; 2.2 and 3.3 respectively). Breeders in the treatment group annually consumed a mean quantity of 45 kg of the standard breeder diet for the farm which was composed of chicken, beef and fish. The standard diet was supplemented with approximately 11 g oxytetracycline, 2.5 g Virginiamycin, 57 g vitamins, 1 kg protein balanced with free amino acids and 0.5 kg of fat low in stearic acid (4%), oleic acid (13%), linolenic acid (2%) but high in n-3 fatty acids (34%). Supplemented fat comprised approximately 10% of the total dietary fat. The control pen was fed the standard breeder diet and annually consumed a mean quantity of 59 kg of the feed without supplement.

Fatty Acid Determinations

Lipids were extracted from yolk, the polar fraction separated by silicic 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. Quantities of each component were determined by relationship of its peak area to that of the methyl heptadecanoate internal standard. Ninety-nine percent (99%) of the long chain fatty acids detected had chain lengths from 16 to 22 carbons. This group of 10 polar fraction fatty acids were designated the AEYLC fatty acids. Preliminary tests showed a close correlation between fatty acid consentrations in the polar and neutral fraction but concentrations of the n-3 group were higher in the polar fraction and, consequently, changes in concentration easier to detect. The common name, systematic name, abbreviation (chain length:number of double bonds) and (position of first double bond from the methyl end) are given (Table 1). They are abbreviated in the text by giving the chain length and the degree of unsaturation but the position of the first double bond from the methyl end is omitted. For example linoleic acid is C18:2.

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) |

Statistics

Statistics were performed using the SAS system, OS/2 version (SAS institute Cary North Carolina) with the assistance of the Station Statistician, Dr Raymon Littell.

RESULTS

Egg Morphometry

Egg volumes ranged from 54.4 cc to 91.1 cc with a mean value of 71.9 cc. Measured egg volumes deviated from calculated egg volumes (calculated for a prolate spheroid with the same length-width dimensions) by from -15.2% to +22.6%. Length/width ranged from 1.48 to 1.89 with a mean value of 1.71. Clutch size and length/width were not correlated with fertility ($r < 0.10$). Egg volume was strongly correlated with fertility ($r=0.61$; $r^2=0.37$). Deviation of measured egg volume from calculated volume had a weak association with fertility ($r=0.22$;$r^2=0.05$).

Fatty Acid Profiles of High and Low Fertility Clutches

With the exception of C16, palmitic acid, all AEYLC fatty acids concentrations from high-fertility clutches were significantly different ($p<0.05$) from the concentrations in the low-fertility clutches (Figs 1,2,3,4). Concentrations of C16:1, palmitoleic acid, the only member of the n-7 group of fatty acids, was found to be significantly higher in the high fertility clutches. Concentrations of C18:1, oleic acid, the only member of the n-9 family, were significantly lower in high fertility clutches. Significant differences were not detected for the n-6 (C18:2, C20:4) and n-3 (C18:3, C20:5, C22:5, C22:6) groups of fatty acids. Low fertility clutches had higher concentrations of 5 of the AEYLC fatty acids: C18; C18:1; C18:2; C20:5; C22:6. Regression analysis of the data indicated that increasing concentrations of C18:2, linoleic acid beyond 7.2%, had the strongest negative correlation with clutch fertility for all eggs in the data set where clutch fertility was known ($N=72$, $r=-0.78$, $r^2=0.61$). The combined concentrations of C18 (stearic) and C18:2 (linoleic) had the strongest negative correlation with egg-fertility of any combinations of AEYLC fatty acids ($N=72$, $r=-0.86$, $r^2=0.74$). These two fatty acids, C18+C18:2, were designated the AEYLC infertile fatty acids (Fig 4). The remaining 8 acids (C16, C16:1, C18:3, C20:4, C20:5, C22:5, C22:6) were designated as the fertile

AEYLC fatty acids and had a positive correlation with egg-fertility (N=72, r=0.86, $r^2$=0.74). The fertile AEYLC fatty acids/infertile AEYLC fatty acids ratio [(fertile/infertile) X 100] was designated the "finratio" and its correlation with clutch fertility determined (N=72, r=0.81, $r^2$=0.66).

Nutrient Therapy

AEYLC fatty acid concentrations in eggs from the treated animals (treated eggs) were compared with eggs from the untreated control pen (untreated eggs). Treated eggs had a significantly higher egg fertility rate (86% versus 77%) and a very different fatty acid profile from the untreated pen (Figs 1 - 4). Eggs from treated animals were significantly smaller than eggs from untreated animals and percent of the egg represented by egg-membrane was significantly less in the treated eggs. There were no significant differences in egg volume, length/width, deviation of measured volume from calculated volume or percent of the egg represented by shell. Linoleic acid, C18:2 was the only fatty acid concentration that was significantly decreased in treated eggs. The magnitude of the change (62% decrease, 7.5 % of the total AEYLC fatty acids) was approximately three times that of any other fatty acid (Fig 3). Statistically significant increases in concentrations were found for 5 fatty acids in treated eggs (%increase, % of AEYLC fatty acids): C18:3 (173%, 0.69% ); C20:4 (39%, 2.9%); C20:5 (304%, 2.46%); C22:5 (46%, 0.26%); C22:6 (141%, 2.4%). The n-3 group of AEYLC fatty acids were significantly increased (171%, 5.8%) and the n-6 group significantly decreased (26%, 5.0%) in the treated eggs. Infertile AEYLC fatty acids were significantly decreased (38%) and fertile AEYLC fatty acids significantly increased (10%). The finratio significantly increased (76%).

Effect of Nutrient Therapy on Egg Hatch-Rates 1986 - 1992

Statistics for nutrient therapy on egg hatch-rates for all treated and control-untreated farms from 1986 through 1992 were studied. The treatment period was for 1 year. There were 9,255 individual breeder treatments (1 breeder treated for 1 year) and 21,215 untreated controls. The treated animals produced 125,486 eggs and 53,328 hatchlings for an egg yield of 13.6 per breeder, a hatch-rate of 42.5% and a hatchling yield of 5.8 hatchlings per breeder. The untreated controls produced 150,872 eggs and 50,273 hatchlings for an egg yield of 13.6 per breeder, a hatch-rate of 33.3% and a hatchling yield of 2.37 per breeder.

## DISCUSSION

Previous studies have clearly indicated that many reproductive parameters of the American alligator are affected by diet (1,2). Nest rate, egg yield, egg fertility, hatch rate and hatchling yield per female have consistently been shown to be significantly increased by nutrient therapy (1,2,3). The summary of 6 years of statistics reported here involved over 275,000 eggs. The effect of preconceptual nutrient therapy on egg-fertility could not be accurately determined but hatch-rate was increased 28%. Egg-fertility was increased by 12% and hatch-rate increased by 19% for the present study on Farm H. The increased hatch-rate on Farm H was also due to a 16% increase in embryo survival in addition to 12% more fertile eggs. Other studies by our laboratory have shown that nutrient therapy produced a 17% increase in the percentage of embryos that survived and an increase in the stage of development reached before embryonic death (1). The present study on egg-fertility attempts to determine dietary constituents that strongly affect that parameter and embryo survival.

The close association of dietary fat with egg fertility suggested that nutrient therapy which focused on dietary fat and lipid metabolism could be successful in improving egg-fertility. The present results show that there are statistically significant differences in the AEYLC fatty acid concentrations between high-fertility and low-fertility eggs collected from widely separated wild areas and from breeding pens on farms. It also shows that nutrient therapy changes the fatty acid profile of treated eggs to more closely resemble the fatty acid profile of high fertility eggs (Figs 1 - 4). Nutrient therapy also seemed to affect the morphometry of the eggs from treated animals. Treated eggs were significantly smaller than untreated eggs and percent of the egg represented by egg-membrane was significantly less for treated eggs. The percent of egg represented by shell, length/width and deviation of measured from calculated volume for treated eggs were not significantly different from untreated eggs.

Some AEYLC fatty acids are more highly correlated with infertility. Surprisingly C18:2, linoleic acid, (considered to be the first "essential fatty acid") in concentrations greater than 7% is strongly associated with egg-infertility and C18, stearic acid, in concentrations greater than 9% is also associated with egg-infertility. When C18, stearic acid and 18:2, linolenic acid concentrations are combined the strongest correlation with infertility is observed at combined concentrations greater than 15%. Fatty acids most strongly associated with fertility in high concentrations were C18:3, linolenic, C20:4 arachidonic and C22:5, clupanodonic acid, although all AEYLC fatty acids are probably needed at some concentration.

The results of these studies show that egg quality can be evaluated for a pen using a relatively small sample and that nutrient therapy can be used to increase pen fertility. The present findings also suggest ways of further improving nutrient therapy.

## ACKNOWLEDGEMENTS

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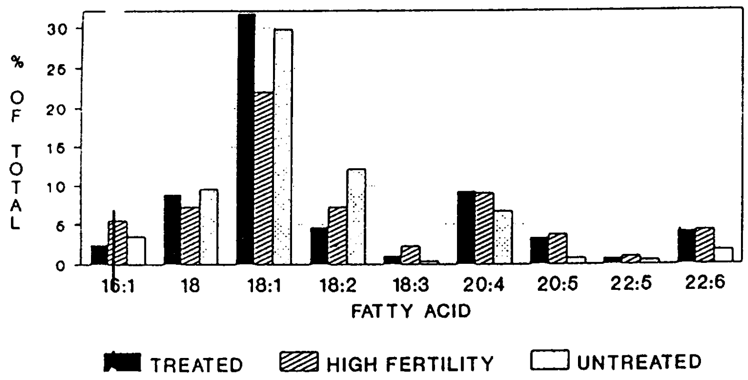
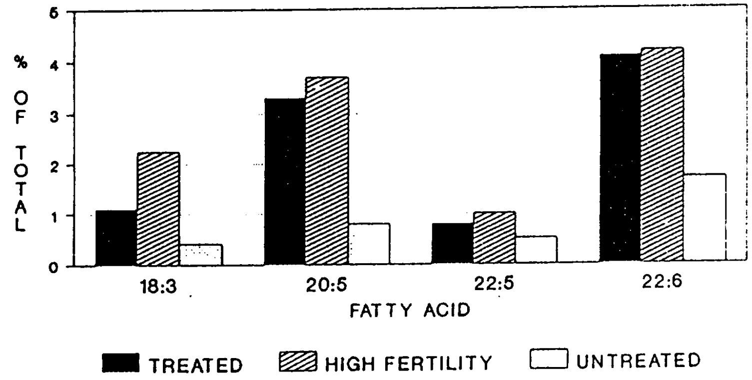
Fig 1 YOLK FATTY ACID PROFILE OF HIGH FERTILIY ALLIGATOR EGGS COMPARED WITH EGGS FROM TREATED & UNTREATED FEMALES



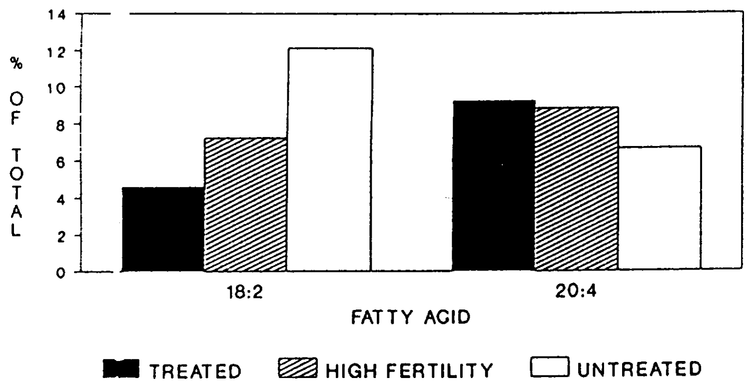

TREATED (FERT = 86%)
HIGH FERTILITY (FERT > 86%)
UNTREATED (FERT = 77%)

Fig 2 YOLK n-3 FATTY ACIDS OF HIGH FERTILITY ALLIGATOR EGGS COMPARED WITH EGGS FROM TREATED & UNTREATED FEMALES



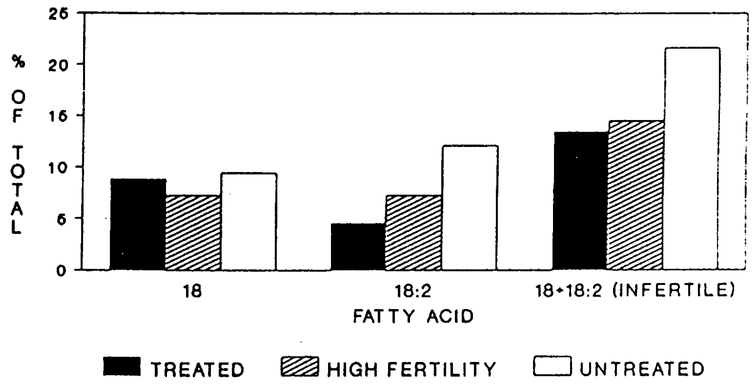

TREATED (FERT = 86%)
HIGH FERTILITY (FERT > 86%)
UNTREATED (FERT = 77%)

Fig 3 YOLK n-6 FATTY ACIDS OF HIGH FERTILITY ALLIGATOR EGGS COMPARED WITH EGGS FROM TREATED & UNTREATED FEMALES




TREATED (FERT = 86%)
HIGH FERTILITY (FERT > 86%)
UNTREATED (FERT = 77%)

Fig 4 ALLIGATOR EGG YOLK INFERTILE FATTY ACIDS HIGH FERTILITY EGGS COMPARED WITH EGGS FROM TREATED & UNTREATED FEMALES




TREATED (FERT = 86%)
HIGH FERTILITY (FERT > 86%)
UNTREATED (FERT = 77%)