

RESEARCH PROGRESS REPORT — January 2019

Research Sponsor: Louisiana Department of Wildlife and Fisheries, Coastal and Non-Game Resources Division (Alligator Resource Funds)

Project Title: Alligator Husbandry Research

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2018 Research Results**I. Effects of fat-soluble vitamins on growth, health and skin quality of alligator**

On November 1, 2017 a feeding trial was initiated to identify effects on alligator growth and health of removing fat-soluble vitamin supplements from a compounded (commercially manufactured) diet.

The study design was as follows:

- 96 alligators (hatched August 2017)
- Four diets — a control diet with complete vitamin supplementation and three experimental diets with no supplemental vitamin A, vitamin D, or vitamin E
- Alligators were fed for 10 months (November 2017– August 2018)
- Four animals from each treatment were sampled at 2.5-month intervals (January, March, May, and August 2018) for necropsy and histopathological examination at the LSU veterinary school to determine the effects of dietary treatment on selected organs and tissues — i.e., brain (cerebrum, cerebellum, brainstem, choroid plexus), bone (femur, vertebra), conjunctiva, esophagus, eye, heart, kidney, liver, lung, muscle (skeletal), pancreas, skin, spleen, thymus, thyroid gland, and trachea

Observations of veterinary pathologists are presented in an Excel file accompanying this report. Removal of vitamin A, D, and E supplements from the test diets did not produce identifiable effects on alligator organ and tissue health, as determined by necropsy and histopathological examination, during 10 months of treatment.

II. Effects of atmospheric ammonia on health of juvenile alligator

An experiment was conducted in early 2018 to identify effects of airborne ammonia on the health of juvenile alligator.

The experimental design was as follows:

- 16 alligators (hatched August 2017)
- Four treatments — exposure to 0, 50, 100, or 200 ppm atmospheric ammonia for 48 hours
- All animals subjected to necropsy and histopathological examination at the LSU veterinary school to determine the effects of ammonia on selected organs and tissues

Necropsies and histopathological examinations by veterinary pathologists found no differences among treatment groups, indicating that exposure to atmospheric ammonia for 48 hours at concentrations up to 200 ppm produced no observable effect on alligator tissues.

III. Effects of hyperoxic conditions during egg incubation on umbilical scarring

An experiment was initiated June 22, 2017 to determine the effects of environmental oxygen concentrations during egg incubation on umbilical scarring of alligators raised to market size.

Design of the experiment was as follows:

- 300 eggs (10 clutches of 30 eggs each)
- Three treatments — 21% atmospheric oxygen (ambient air), 25% oxygen, or 28% oxygen
- Incubate at 88 F and 90%+ humidity with sphagnum moss on all sides of eggs
- Upon hatchling's emergence from the egg, umbilical area was measured and photographed (Figure 1) and ID chip was placed for tracking
- After three days in the incubator, post-hatch, hatchlings were transferred to a commercial farm for grow-out to market size.

Results will be available at the end of the grow-out period, likely in late 2019.



Figure 1. The umbilical opening of each hatchling was measured and photographed, and a chip ID was inserted under the skin behind the head to allow tracking of individual animals. These data will be used to identify possible effects of hyperoxic incubation conditions on umbilical scarring at harvest.

IV. Estimation of minimum dietary requirements for essential amino acids, lysine and methionine

Growth trials to estimate minimum dietary requirements for lysine and methionine have been completed.

Design of the experiments was as follows:

- 144 alligators (hatched August 2018) stocked eight per tank in 18, 3-m² plastic tanks
- Six dietary treatments — three levels of lysine and three levels of methionine ranging above and below the expected dietary requirement for each amino acid
- Six diets assigned to 18 tanks of alligators (three tanks per diet); fed for 90 days.

Analytical work (qPCR) is in progress to determine effects of dietary treatments on gene expression. If successful, results of qPCR could be helpful in determining minimum requirements for other dietary essential amino acids in less time than lengthy growth trials.

V. Determination of amino acid composition of muscle and normal-scale vs. double-scale skin

Laboratory analyses to determine if the amino acid composition of normal-scale skin differs from that of double-scale skin were conducted in 2018. The amino acid composition of alligator muscle also was determined for use as a reference protein in future studies.

Design of the study was as follows:

- Six skins with attached muscle were obtained from three farms (two skins per farm) — one normal skin and one double-scale skin from each farm
- Amino acid composition of patches of double-scale skin and patches of normal-scale skin was determined for comparison (Table 1)
- Amino acid composition of muscle was determined (Table 1)

Table 1. Percentage (mean \pm standard deviation) of amino acids in muscle and skin of alligators without double-scale (C = control) and with double-scale (DS) condition. Means represent the average of muscle and skin samples obtained from three farms. There were no significant differences ($P > 0.05$) in amino acid composition of muscle taken from C or DS animals or amino acid composition of skin taken from C or DS animals.

AMINO ACID	C-Muscle		DS-Muscle		C-Skin		DS-Skin	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Aspartic Acid	4.17	0.89	4.03	0.69	3.82	0.74	3.85	0.69
Glutamic Acid	6.28	1.42	5.80	1.48	5.82	1.18	5.84	1.05
Asparagine	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Serine	2.11	0.48	2.24	0.32	3.00	0.52	3.05	0.49
Glutamine	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Histidine	1.45	0.37	1.36	0.31	0.81	0.23	0.85	0.24
Glycine	5.79	1.65	5.02	1.02	17.33	2.48	16.62	4.35
Threonine	2.26	0.48	2.21	0.40	2.03	0.43	2.08	0.38
Arginine	2.87	0.48	2.74	0.44	3.66	0.53	3.65	0.55
Alanine	4.18	0.66	3.93	0.61	6.36	0.95	6.16	1.25
Tyrosine	0.99	0.27	1.01	0.19	1.06	0.33	1.11	0.36
Cysteine	4.56	1.59	4.13	1.16	13.60	2.26	12.66	3.52
Valine	2.48	0.51	2.34	0.37	1.86	0.38	1.85	0.41
Methionine	1.11	0.29	1.08	0.21	0.80	0.22	0.81	0.20
Tryptophan	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Phenylalanine	1.51	0.32	1.43	0.24	1.35	0.26	1.35	0.22
Isoleucine	2.17	0.49	2.08	0.39	1.47	0.40	1.47	0.40
Leucine	3.45	0.77	3.23	0.81	2.57	0.62	2.57	0.60
Lysine	8.21	3.23	8.54	4.29	7.85	3.28	7.49	2.15
Hydroxyproline	0.77	0.53	0.74	0.38	3.00	1.00	2.83	1.38
Proline	2.22	0.50	2.05	0.32	5.20	0.71	4.77	1.31
Sum of AA	56.57	8.46	53.97	6.59	81.57	10.29	79.02	12.58

The lack of significant difference between the amino acid composition of normal skins and double-scale skins suggests that amino acid composition of the skin is unlikely to be a relevant factor affecting development of the double-scale condition.

Submitted 11 January 2019 by:

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