

CARP CAKES: ANOTHER FISH ALTERNATIVE

April Braddy, BS,^{1,2} Andrew Clarke, PhD,³ Duane Chapman, MS,⁴ Kevin McGraw, PhD,⁵ Kevin Fritsche, PhD,⁶ Ellen Dierenfeld, PhD¹*

¹*Department of Animal Health and Nutrition, Saint Louis Zoo, St. Louis, MO;* ²*Department of Agriculture, Murray State University, Murray, KY;* ³*Department of Food Science, University of Missouri-Columbia, Columbia, MO;* ⁴*U.S. Geological Survey-CERC, Columbia, MO;* ⁵*School of Life Sciences, Arizona State University, Tempe, AZ;* ⁶*Department of Animal Science, University of Missouri – Columbia, Columbia, MO*

Extended Abstract

Introduction

Silver carp (*Hypophthalmichthys molitrix*) and bighead carp (*H. nobilis*) are invasive species that pose a threat of displacing and lowering the abundance of native fishes, mussels and invertebrates.¹⁰ The Mississippi Interstate Cooperative Resource Association (MICRA) considers bighead and silver carp the most important aquatic nuisance species in the basin.⁴ On the Missouri, Mississippi and Illinois rivers, bighead and silver carp populations have the potential to double in a year.¹⁴ Environmental threats from high populations of silver carp include competition for food with native planktivores and larval fish of nearly all species, and also changes in water quality.¹¹ Bighead carp can reach sizes up to 50 kg and silver carp can reach up to 27.3 kg.¹⁴

Since carp are prolific and grow to large sizes they have the potential to provide an economical alternative as food for piscivorous species in zoos, thus reducing harvest of marine fish and assisting in clearing non-native fishes from local waterways.

The development of silver-carp-based diets for zoo animals was initiated. Since the size of silver carp precluded simply feeding the whole fish to the animals, development of a product made from ground, whole, uncooked carp was begun. Early steps in the project involved collaborating with the University of Missouri-Columbia, U.S. Geological Survey, Missouri Department of Natural Resources, Missouri Department of Conservation, and the Native Fish Conservancy to create a recipe mimicking the size and nutritional composition of whole fish. Whole silver carp were ground and formed into a processed “cake” using a cold gel set process developed by food scientist Dr. Andrew Clarke of the University of Missouri-Colombia.⁵ Fat-soluble vitamins A and E were extracted and proximate nutrient composition (water, protein, fat, ash) analyzed, along with mineral and fatty acid profiles of cold set gel carp product, before the addition of Vitamins B and E. In addition to nutrient content, carp cake texture is extremely important for acceptability by piscivores; hence measurements were taken for cohesion, as well as “tossability” (for feeding program application). Prototypes were soaked in water for up to two hours to ensure they would remain cohesive. Supplemental levels of B vitamins and vitamin E will be added to a final prototype. The final product will be tested in a pilot feeding trial with penguins, pelicans, and sea lions at the St. Louis Zoo and other participating facilities in 2007. Palatability and intake, physiological status of the animals (including weights and body condition), and

circulating blood nutrient concentrations will be monitored on each of the animals involved in the pilot feed study to determine product efficacy.

Methods

Diet Survey & Analysis

In order to evaluate how much fish is fed to animals in American Zoo and Aquarium Association accredited zoos and aquariums in North America, a survey was distributed electronically through an aquariums list-serve. The survey requested information on annual tonnage and type of fish used.

Nutrition Analysis. Three whole silver carp were caught from the Missouri River and individually ground. Aliquots were freeze-dried to determine water content, and the dried, ground samples were sent for proximate analysis (crude protein, crude fat, crude fiber, ash) and minerals to Dairy One Forage Lab (Ithaca, NY).

Fat Soluble Vitamins. Aliquots of puree from the three silver carp were extracted using standardized lab protocols for whole meats.¹ Extracts were stored at -20° C until shipment to Arizona State University, Tempe, AZ where they were analyzed via HPLC following the methods of McGraw.¹² Pigment extracts were injected into a Waters Alliance 2695 HPLC system (Waters Corporation, Milford, MA) fitted with a Waters YMC Carotenoid 5.0 μ m column (4.6 mm x 250 mm) and a built-in column heater set at 30° C. A three-step gradient solvent system was used to analyze both xanthophylls and carotenes in a single run, at a constant flow rate of 1.2 ml min⁻¹: first, isocratic elution with 42:42:16 (v/v/v) methanol : acetonitrile : dichloromethane for 11 min, followed by a linear gradient up to 42:23:35 (v/v/v) methanol : acetonitrile : dichloromethane through 21 min, held isocratically at this condition until 30 min, and finishing with a return to the initial isocratic condition from 30 to 48 min. Data were collected from 250 to 600 nm using a Waters 2996 photodiode array detector. Pigments were identified by comparing their respective retention times and absorbance maxima (λ_{\max}) to those of reference carotenoids run as external standards.

Fatty Acids. Frozen samples of the pureed carp were sent to the University of Missouri-Columbia for determination of fatty acid content. Total lipids were extracted with chloroform:methanol (2:1 v/v) as described by Folch⁸ after samples were homogenized in 10 mM EDTA. The fatty acid profile of the carp cakes were determined as described by Sukhija and Palmquist (1988) Lipid extracts were methylated with freshly made 5% methanolic HCl.¹³ The resulting fatty acid methyl esters were extracted with benzene. Pigments and residual water were removed from the samples with the addition of 0.1 g each of anhydrous sodium sulfate and activated charcoal to each sample. Fatty acid methyl esters were analyzed by gas chromatography using an HP 5890A instrument with a 30-meter capillary column (Omegawax 250; Supelco, Bellefonte, PA). Individual fatty acids were identified using retention times of standards (i.e., Omegawax, PUFA-II, PUFA-I; Supelco). Fatty acid data are expressed as a percentage of total fatty acids in the sample (i.e., g/100g).

Thiamin and Vitamin E Concentrations. Silver carp puree was tested for both thiamin and vitamin E. Sample A was only carp puree. Samples B-D were carp puree with the addition of 0.2, 0.4, and 0.8g of Thiamin-E vitamin supplement paste, formulated for whole fish, from Stuart Products, Inc. The final sample was a tube of Thiamin-E vitamin supplement paste from Stuart Products, Inc. Bedford, TX. Samples A-D each consisted of 200g of ground whole carp. The samples were sent to Medallion Labs, Minneapolis, MN, to be analyzed for thiamin and vitamin E.

Results and Discussion

Data from the 43 institutions who replied to the survey are included in this study. The annual tonnage is given in Table 1. Herring (*Clupea harengus*) and capelin (*Mallotus villosus*) were the fish most used at the zoos and aquariums surveyed, followed by mackerel (*Scomberomorus scombrus*), sardines (species not indicated), and silversides (*Menidia andens*). The total amount of fish used in the previous year in just the zoos surveyed was > 1,600,000 metric tons.

The initial analyses of the proximate chemical composition, calculated vitamin A and E content, and mineral concentrations in whole carp (Table 2) were most similar to those values in herring. Herring had a proximate chemical composition and mineral concentration of: protein 44.2-70.2%, fat 16.4-38.3%, calcium 1.56-1.85%, phosphorus 1.00-2.13%, and ash 7.3-11.9%.² The mineral values were as expected for whole fish with the possible exception of iron; the levels measured were in excess compared to herring.² The vitamin E content of silver carp puree, even after exposure to air and possible oxidation due to pureeing, was very high. However, vitamin A levels seemed to be low compared to most whole fish.⁶

Carp are known to be relatively high in polyunsaturated fatty acids, including omega-6 fatty acids.⁷ Our analysis confirms that carp are high in omega-6 fatty acids. Molar percentages of fatty acids in silver carp (n=3) are found in Table 3.

It is known that fish-based diets require supplementation with vitamin E and thiamin (B1).² Many fish are known to have substantial amounts of thiaminase present in their tissues.³ Thiaminase is an enzyme that destroys or inactivates thiamin.⁹ Clearly, the levels of vitamin E and thiamin observed in the supplemented fish were not what was expected. The analyzed results of thiamin and vitamin E testing from Medallion Labs, Minneapolis, MN, are in an as is basis. The calculated results were the amount of thiamin and vitamin E added to the 200g of ground whole carp. This supplement may not be the most suitable one to use since only 73% of the guaranteed vitamin E and only 31% of the expected thiamin was observed in the supplemental paste, formulated for whole fish, from Stuart Products, Inc. It is also very likely that thiaminase was breaking down the thiamin in the carp puree. These results indicate that higher concentrations or more stable forms of thiamin and vitamin E need to be used.

LITERATURE CITED

1. Barker, D., M. P. Fitzpatrick, and E.S. Dierenfeld, E.S. 1998. Nutrient composition of selected whole invertebrates. Zoo Biol. 17:223-134.

2. Bernard, Joni B. and Mary E. Allen. 1997. Feeding captive piscivorous animals: nutritional aspects of fish as food. AZA Nutritional Advisory Group Handbook. Fact Sheet 005.
3. Breen, R.G., W.E. Carlson, and C.A. Evans. 1942. The inactivation of Vitamin B1 in diets containing whole fish. *J. Nutrition*. 23: 165-174.
4. Chapman, D.C., and Wang, N., 2006. Notes on the translation and use of "a study of the early development of grass carp, black carp, silver carp and bighead carp in the Yangtze River, China," chap.1 of Chapman, D.C., ed., *Early development of four cyprinids native to the Yangtze River, China*: U.S. Geological Survey, Data Series 239, p. 1-9.
5. Clarke, Andrew D., Rajitha Kolli, Ellen S. Dierenfeld, Kevin L. Fritsche, and Duane C. Chapman. 2007. Development of Restructured Silver Carp Patties for Feeding Exotic Animals. 2007 Missouri Natural Resources Conference Invasive Species Workshop.
6. Dierenfeld, E.S., N. Katz, J. Pearson, F. Murru, and E.D. Asper. 1991. Retinol and alpha-Tocopherol concentrations in whole fish commonly fed in zoos and aquariums. *Zoo Biology* 10:119-125.
7. Domaizon, I., C. Desvillettes, D. Debroas and G. Bourdier. 2000. Influence of zooplankton and phytoplankton on the fatty acid composition of digesta and tissue lipids of silver carp: mesocosm experiment. *Journal of Fish Biology*. 57:417-432.
8. Folch, J, M. Less, and GH Stanley. 1957. A simplified method for the isolation and purification of total lipids from animal tissues. *J Biol Chem*. 226: 497-509.
9. Fujita, A. 1954. Thiaminase. *Advances in Enzymology*. 389-421.
10. Koel, Todd M., Kevin S. Irons, and Eric Ratcliff. 2000. Asian carp invasion of the upper Mississippi river system. Retrieved June 13, 2007 from http://www.umesc.usgs.gov/reports_publications/psrs/psr_2000_05.html.
11. Kolar, C.S., D.C. Chapman, W.R. Courtenay, C.M Housel, J.D. Williams, and D.P. Jennings. 2007. *Bigheaded Carps: A Biological Synopsis and Environmental Risk Assessment*. American Fisheries Society. 204 pp.
12. McGraw, K. J., P. M. Nolan, and O. L. Crino. 2006. Carotenoid accumulation strategies for becoming a colorful house finch: analyses of plasma and liver pigments in wild molting birds. *Funct. Ecol*. 20:678-688.
13. Sukhija, PS and DL Palmquist. 1988. Rapid method for determination of total fatty acid content and composition of feedstuffs and feces. *J Agric Food Chem*. 36: 1202-1206.
14. U.S. Fish and Wildlife Services. (2004). Asian Carp: Huge Fish with Huge Impacts. In *Asian Carp - An Aquatic Nuisance Species*. Retrieved June 13, 2007, from <http://www.asiancarp.org/Documents/AsianCarp.pdf>

Table 1. Annual tonnage of fish used by American Zoo and Aquarium Association accredited zoos and aquariums.

Type of Fish	Metric tons of fish used
Herring	500658
Mackerel	40231
Capelin	950681
Marine Smelt	20021
Lake Smelt	15071
Sardines	50078
Trout	5054
Silversides	20032
Salmon	12014
Misc.*	100
Total	1613940

* The total tonnage of squid, mahi mahi, mullet, hoki, sand eels, anchovies, krill, Pollock, bonito, pompano, sand lance, shrimp, clam strips, scallops, bluefish, butterfish, blue runner.

Table 2. Proximate nutrient composition, minerals, and vitamins A and E of silver carp (*Hypophthalmichthys molitrix*). (Mean \pm standard deviation (SD); all nutrients except water on a dry matter basis.)

Nutrients	n=3	
	Mean	SD
Proximate Nutrients		
Water, %	69.35	± 2.88
Crude Protein, %	61.53	± 4.63
Crude Fat, %	23.20	± 5.82
Ash, %	17.89	± 2.14
Minerals		
Calcium, %	2.96	± 1.13
Phosphorus, %	2.41	± 0.36
Magnesium, %	0.13	± 0.02
Potassium, %	0.67	± 0.07
Sodium, %	0.44	± 0.09
Iron, mg/kg	1230.33	± 374.53
Zinc, mg/kg	50.33	± 6.11
Copper, mg/kg	3.33	± 0.58
Manganese, mg/kg	144.33	± 48.43
Molybdenum, mg/kg	0.30	± 0.14
Sulfur, %	0.55	± 0.04
Chloride, %	0.50	± 0.10
Cobalt, mg/kg	1.59	± 0.13
Vitamins and Carotenoids		
Vitamin A, IU/kg	1448.05	± 104.13
Vitamin E, IU/kg	686.92	± 133.08
Carotenoids, $\mu\text{g/g}$	5.32	± 1.75

Table 3. Mean fatty acid concentrations (\pm standard deviation (SD)) of silver carp (*Hypophthalmichthys molitrix*). (n=3) Results are reported in g/100g.

Fatty acids	Mean	SD
14:0	9.68	± 0.41
16:0	28.97	± 3.90
16:1	19.39	± 1.60
18:0	3.45	± 0.47
18:1n7&9	18.54	± 5.85
18:2n6	3.49	± 1.17
18:3n6	0.00	0
18:3n3	5.16	± 3.09
18:4n3	1.02	± 0.78
20:2n6	0.23	± 0.20
20:3n6	0.22	± 0.22
20:4n6	1.42	± 0.95
20:5n3 (EPA)	4.74	± 3.19
22:1n9	0.00	0
22:4n6	0.00	0
22:4n3	0.45	± 0.33
22:5n6	0.00	0
22:5n3	0.97	± 0.61
<u>22:6n3 (DHA)</u>	2.27	± 1.44

Table 4. Observed thiamin and vitamin E concentrations in carp puree versus the calculated values based on known supplementation levels. Results are reported on an as is basis.

Sample	Calculated	Analyzed
	vitamin E (IU/100g)	
Carp puree A	0	0.569
Carp puree B	20	7.96
Carp puree C	40	15.5
Carp puree D	80	24.2
Thiamin-E Paste	10000	7320
	Thiamin (mg/100g)	
Carp puree A	0	<0.05
Carp puree B	10	0.08
Carp puree C	20	0.13
Carp puree D	40	3.67
Thiamin-E Paste	5000	1572