PD/A CRSP Sixteenth
Annual Technical Report

Nutritional Contribution of Natural and Supplemental Foods for Nile Tilapia: Stable Carbon Isotope Analysis

Eighth Work Plan, Kenya Research 3A (KR3A)
Progress Report

Rebecca Lochmann and Peter Perschbacher
Department of Aquaculture and Fisheries
University of Arkansas at Pine Bluff
Pine Bluff, Arkansas, USA

Abstract

Stable carbon isotope analysis is a useful technique to obtain quantitative estimates of the relative contributions of different food sources to the nutrition of aquatic animals in ponds. This technique is being used to obtain quantitative estimates of the contribution of natural and supplemental feeds to the nutrition of tilapia in ponds in Sagana, Kenya. Results can be used to adjust feeding/fertilization practices and minimize feed costs while maximizing fish production. Samples of *Oreochromis niloticus*, *Clarias*, chemical fertilizers (DAP and urea), rice bran, plankton, and mud taken from ponds in Sagana at three times during the study (initial, midpoint, final) have been submitted to a commercial lab for carbon isotope analysis. Results for initial and some of the midpoint samples are summarized and discussed in this report. The most distinct trend in the isotope data was the more positive values for plankton, *Clarias*, and *O. niloticus* found in Treatment 1 versus Treatments 2 through 4 for both initial and midpoint samples. Possible reasons for this trend are discussed in light of experimental and non-experimental variables. A more comprehensive discussion of the effects of various nutrient inputs on the production of *O. niloticus* and *Clarias* will be possible once the remaining isotope data are obtained.

Introduction

Stable carbon isotope analysis is a useful technique to obtain quantitative estimates of the relative contributions of different food sources to the nutrition of aquatic animals in ponds (e.g., Schroeder, 1983; Anderson et al., 1987; Lochmann and Phillips, 1996). Presumably, isotope ratios of the fish will resemble those of the food(s) they assimilate most. In the present study, stable carbon isotopic analysis is being used to obtain quantitative estimates of the contribution of natural and supplemental feeds to the nutrition of tilapia in ponds in Sagana, Kenya. Results can be used to adjust feeding/fertilization practices and minimize feed costs while maximizing fish production.

To date, selected samples of *Oreochromis*, *Clarias*, chemical fertilizers (diammonium phosphate (DAP) and urea), rice bran, plankton, and mud taken from ponds in Sagana at three times during the study (initial, midpoint, final) have been submitted to a commercial lab for carbon isotope analysis. Since not all data for midpoint and final samples have been received, this report is a summary and preliminary discussion of results for the initial and some midpoint samples.

Methods and Materials

Several months prior to collection of initial samples, *O. niloticus* and *Clarias* were fed a conditioning diet containing corn to increase the isotopic resemblance of the fish to corn (-14‰). Experimental treatments for the pond feeding trial were:

1) Urea and DAP to provide 16 kg N ha^{-1} wk^{-1} and 4 kg P ha^{-1} wk^{-1};
2) Urea and DAP applied to give 8 kg N and 2 kg P ha^{-1} wk^{-1}, plus rice bran fed at 60 kg ha^{-1} d^{-1};
3) Rice bran fed at 120 kg ha^{-1} d^{-1};
4) Rice bran as in treatment 3 and fertilizer as in treatment 2.

The major components of the pond system assumed to contribute to the nutritional status of *O. niloticus* and *Clarias* in this feeding study were sampled monthly throughout the study. Samples from three periods (initial, midpoint, and final) were processed and subjected to isotope analysis. Initially, a total of ten fish—five *O. niloticus* and five *Clarias*—were collected from a single pond at the Sagana research site, Sagana, Kenya. Initial fish samples were not pooled so that the variability of isotope ratios among individuals could be determined. Initial pooled samples of plankton and mud were collected from each of the 12 study ponds, as well as samples of chemical fertilizers (DAP, urea) and rice bran used as supplemental feed. All samples were processed as described previously (Lochmann and Phillips, 1996), except that carbonates were removed from mud samples prior to lyophilization, and DAP and urea samples were not processed prior to analysis. Samples collected from the midpoint and final periods were the same as described for initial samples except that a pooled sample of *O. niloticus* and a pooled sample of *Clarias* (each pooled sample consisting of two individuals) was collected from each pond. All samples were sent to a commercial laboratory (Coastal Science Laboratories, Inc., Austin, Texas) for stable carbon isotope analysis using a micromass isotope ratio mass spectrometer (Anderson et al., 1987).

Results

The mean isotope ratio ($\delta^{13}C$) of initial plankton samples in Treatment 1 was significantly more positive than the mean isotope ratios of plankton in Treatments 2 through 4 (Table 1). The mean $\delta^{13}C$ of initial mud samples did not differ among treatments (Table 1). The mean initial $\delta^{13}C$ of *Clarias* was more variable and approximately 3‰ more negative than that of *O. niloticus* (Table 1). The $\delta^{13}C$ of initial urea was -53.6‰ and

Table 1. Initial stable carbon isotope values ($\delta^{13}C$) of nutrient inputs and pond components in a feeding trial with *O. niloticus* and *Clarias* in Sagana, Kenya. Values are means of three replicates. The mean initial isotope values for fish (N = 5 individual fish) were: *O. niloticus* (-16.61 ± 0.70) and *Clarias* (-19.82 ± 1.19).

Treatment	$\delta^{13}C$ (‰) ± S.D.	
	Plankton	*Mud*
1. Chemical fertilizer: Urea (16 kg N ha^{-1} wk^{-1}) and DAP (4 kg P ha^{-1} wk^{-1})	-17.27 ± 0.47[a]	-13.53 ± 1.39
2. 1/2 of treatment 1	-23.50 ± 1.61[b]	-13.08 ± 0.63
3. Rice bran only (120 kg ha^{-1} d^{-1})	-26.27 ± 2.49[b]	-13.33 ± 0.72
4. Rice bran (as treatment 3) and Chemical fertilizer (as treatment 2)	-26.83 ± 2.49[b]	-12.30 ± 0.54

[a,b] Means in columns with different letters are significantly different ($P < 0.05$) according to Fisher's Least Significant Difference test.

Table 2. Midpoint stable carbon isotope values ($\delta^{13}C$) of nutrient inputs and pond components in a feeding trial with *O. niloticus* and *Clarias* in Sagana, Kenya. Values are means of three replicates.

Treatment	$\delta^{13}C$ (‰) ± S.D.			
	Tilapia	*Clarias*	*Plankton*	*Mud*
1. Chemical fertilizer: Urea (16 kg N ha^{-1} wk^{-1}) and DAP (4 kg P ha^{-1} wk^{-1})	-17.88 ± 0.86[a]	-22.40 ± 2.08[a]	-22.43 ± 1.79[a]	NA[c]
2. 1/2 of treatment 1	-22.83 ± 0.72[b]	-24.67 ± 1.27[ab]	-26.97 ± 1.16[b]	NA
3. Rice bran only (120 kg ha^{-1} d^{-1})	-22.93 ± 2.39[b]	-26.42 ± 1.23[b]	-29.55 ± 1.23[b]	NA
4. Rice bran (as treatment 3) and Chemical fertilizer (as treatment 2)	-24.03 ± 1.17[b]	-26.83 ± 0.61[b]	-27.83 ± 1.75[b]	NA

[a,b] Means in columns with different letters are significantly different ($P < 0.05$) according to Fisher's Least Significant Difference test.
[c] Isotope data for midpoint mud samples is currently not available.

the mean isotope ratios of DAP and rice bran were very similar: -26.8 and -27.8‰, respectively.

The mean $\delta^{13}C$ of midpoint plankton samples in Treatment 1 was significantly more positive than that of plankton in Treatments 2 through 4 (Table 2), as observed in the initial samples. The mean isotope ratios of *O. niloticus* and *Clarias* followed the same pattern as the plankton, although statistical differences were less pronounced for *Clarias* (Table 2).

The mean isotope ratios of plankton were significantly more negative ($P = .0001$) in all treatments in the midpoint than in the initial and samples (Tables 1 and 2). The mean isotope ratios of *O. niloticus* and *Clarias* followed the same trend (Tables 1 and 2).

DISCUSSION

Corn, which was fed as the conditioning diet prior to collection of initial samples, is isotopically distinct from the rice bran (-27‰) that was used as a supplemental feed in Treatments 2, 3, and 4. The isotope technique is more effective in pinpointing nutritional inputs of an animal when the inputs are isotopically distinct from each other and from the animal itself (Anderson et al., 1987). However, the mean isotope ratios of initial *O. niloticus* (-16.6‰) and *Clarias* (-19.8‰) were not similar to that of corn (-14‰). It is possible that the amount of corn in the conditioning diet was too low to influence the isotope ratios of the fish, the corn was not assimilated well, or the diet was not fed long enough to elicit the desired isotopic effect in the fish.

The most distinct trend in the isotope data was the more positive values for plankton, *Clarias* and *O. niloticus* in Treatment 1 versus Treatments 2 through 4 for both initial and midpoint samples. Treatment 1 did not include rice bran, whereas Treatments 2 through 4 did. The rice bran was consumed directly by the fish, which may explain the increase in isotope values between initial and midpoint samples. However, the isotope values of the plankton in treatment 1 were more positive initially than those of the plankton in the other treatments. This suggests that the result may be due to an undefined pre-treatment effect. Karen Veverica also observed significantly higher chlorophyll *a* concentrations in Treatment 1 of this study vs. other treatments (see Veverica et al., 1999).

Some components other than the inputs identified earlier may have contributed to the isotope ratios of the plankton between initial and midpoint sampling periods. Rice bran was the only experimental input but apparently not the only factor influencing the isotope ratio of the plankton in Treatment 3, as was indicated by the more negative value of the midpoint plankton (-29.6‰) compared to the rice bran (-27.8‰). Also, the isotopic similarity of the DAP (-26.8‰) and the rice bran (-27.8‰) may confound interpretation of results for Treatments 2 and 4, which received both components as pond inputs.

A more comprehensive discussion of the effects of various nutrient inputs on the production of *O. niloticus* and *Clarias* will be possible once the remaining isotope data are obtained.

ANTICIPATED BENEFITS

Production efficiency of *O. niloticus* and *Clarias* can be optimized once the quantitative importance of different nutrients under defined experimental conditions is established using the isotope technique in conjunction with comprehensive production data. Furthermore, the procedures used to define the importance of various components in this aquaculture production system may be modified and applied to other systems in other regions.

ACKNOWLEDGMENTS

The authors wish to thank the staff at Sagana fish farm for collection and processing of samples prior to isotope analysis.

LITERATURE CITED

Anderson, R.K., P.L. Parker, and A. Lawrence, 1987. A $^{13}C/^{12}C$ tracer study of the utilization of presented feed by a commercially important shrimp *Penaeus vannamei* in a pond growout system. J. World Aquacult. Soc., 18:148-155.

Lochmann, R. and H. Phillips, 1996. Stable isotopic evaluation of the relative assimilation of natural and artificial foods by golden shiners (*Notemigonus crysoleucas*) in ponds. J. World Aquacult. Soc., 27:168-177.

Schroeder, G.L., 1983. Stable isotope ratios as naturally occurring tracers in the aquaculture food web. Aquaculture, 30:203-210.

Veverica, K., W. Gichuri, and J. Bowman, 1999. Relative contribution of supplemental feed and inorganic fertilizers in semi-intensive tilapia production. In: K. McElwee, D. Burke, M. Niles, and H. Egna (Editors), Sixteenth Annual Technical Report. Pond Dynamics/ Aquaculture CRSP, Oregon State University, Corvallis, Oregon, pp. 39-42.

Cite as: [Author(s), 1999. Title.] *In*: K. McElwee, D. Burke, M. Niles, and H. Egna (Editors), Sixteenth Annual Technical Report. Pond Dynamics/ Aquaculture CRSP, Oregon State University, Corvallis, Oregon, [pp. ___.]