NUTRITIONAL CONTRIBUTION OF NATURAL AND SUPPLEMENTAL FOODS FOR NILE TILAPIA: STABLE CARBON ISOTOPE ANALYSIS

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ABSTRACT

Stable carbon isotope analysis can be used to obtain quantitative estimates of the relative contributions of different food sources to the nutrition of aquatic animals in ponds. Results can be used to make recommendations for feeding and fertilization practices that will minimize feed costs while maximizing fish production. This technique was used in conjunction with fish gut content analysis to obtain estimates of the contribution of natural and supplemental feeds to the nutrition of *Oreochromis niloticus* and *Clarias gariepinus* in ponds receiving different inputs in Sagana, Kenya. Four combinations of fertilizers and supplemental feed (rice bran) were used as experimental treatments: 1) Urea (16 kg N ha^{-1} wk^{-1}) + DAP (4 kg P ha^{-1} wk^{-1}); 2) Urea (8 kg N ha^{-1} wk^{-1}) + DAP (2 kg P ha^{-1} wk^{-1}) + Rice bran (60 kg ha^{-1} d^{-1}); 3) Rice bran (120 kg ha^{-1} d^{-1}); and 4) Rice bran (120 kg ha^{-1} d^{-1}) +Urea (8 kg N ha^{-1} wk^{-1}) + DAP (2 kg P ha^{-1} wk^{-1}). Samples of *Oreochromis*, *Clarias*, chemical fertilizers (DAP and urea), rice bran, plankton, and mud taken from ponds in Sagana at three times (initial, midpoint, final) during a 143-day feeding trial were analyzed for carbon isotope content. The most distinct trend in the isotope data was the more positive values for plankton, *Oreochromis*, and *Clarias* in treatment 1 versus treatments 2 through 4 for initial, midpoint, and final samples. The addition of rice bran to ponds in treatments 2 through 4 clearly increased fish production relative to ponds where the only inputs were DAP and urea. Gut content analysis indicated that the two most important food categories for *Oreochromis* and *Clarias* in treatments that included rice bran were plankton and rice bran. However, the isotope data did not allow further clarification of the relative nutritional importance of the two categories between treatments because the isotope ratios of plankton and rice bran were not isotopically distinct.

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 isotope ratios of feeds, fertilizers, plankton, and mud were compared to those of *Oreochromis* and *Clarias* co-cultured in ponds receiving different inputs in Sagana, Kenya, during a 143-day feeding trial. We anticipate using the results to optimize nutrient utilization and minimize feed/fertilizer costs for *Oreochromis* production in Kenya and possibly other locations.

METHODS AND MATERIALS

Several months prior to collection of initial samples, *O. niloticus* and *C. gariepinus* were fed a conditioning diet containing corn to increase the isotopic resemblance of the fish to corn (-14‰). Four combinations of fertilizers and supplemental feed (rice bran) were used as experimental treatments:

1) Urea (16 kg N ha^{-1} wk^{-1}) + DAP (4 kg P ha^{-1} wk^{-1});
2) Urea (8 kg N ha^{-1} wk^{-1}) + DAP (2 kg P ha^{-1} wk^{-1}) + Rice bran (60 kg ha^{-1} d^{-1});
3) Rice bran (120 kg ha^{-1} d^{-1}); and
4) Rice Bran (120 kg ha^{-1} d^{-1}) + Urea (8 kg N ha^{-1} wk^{-1}) + DAP (2 kg P ha^{-1} wk^{-1}).

Each treatment was applied to three ponds. The major components of the pond system assumed to contribute to the nutritional status of *Oreochromis* and *Clarias* were sampled monthly throughout the study. The components were: supplemental feed (rice bran), chemical fertilizers (DAP and urea), plankton, and mud. Samples from three periods (initial, midpoint, and final) were processed and subjected to isotope analysis. Initially, five individual *Oreochromis* and *Clarias* (a total of ten fish) were collected from a single pond at Sagana Fish Farm, Sagana, Kenya. Initial fish samples were not pooled to allow determination of variability in isotope ratios among individuals. Initial pooled samples of plankton and mud were collected from each of the 12 study ponds, as well as samples of DAP, urea, and rice bran. 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 *Oreochromis* 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 that of plankton in treatments 2-4 (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 approximately 3‰ more negative than that of *Oreochromis*, and the *Clarias* data was more variable (Table 1). There were no differences in isotope ratios of initial mud samples among treatments. The $\delta^{13}C$ of initial urea was -53.6‰ and those 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 in initial samples. The mean isotope ratios of *Oreochromis* and *Clarias* followed the same pattern as the plankton, although statistical differences were less pronounced for *Clarias* (Table 2). There were no differences in isotope ratios of midpoint mud samples among treatments.

The mean $\delta^{13}C$ of final plankton samples was significantly more positive than that of plankton in treatments 2 through 4 (Table 3), as in initial and midpoint samples. The mean isotope ratios of *Oreochromis* and *Clarias* from treatment 1 were significantly less negative than those of treatments 2 through 4. In addition, the mean isotope ratios of *Oreochromis* and *Clarias* from treatment 2 were significantly less negative than those from treatment 3, while isotope ratios of both species from treatment 4 were between those of fish from treatments 2 and 3 (Table 3). There were no differences in isotope ratios of final mud samples among treatments.

Table 1. Initial stable carbon isotope values ($\delta^{13}C$) of pond components in a feeding trial with *Oreochromis* and *Clarias* in Sagana, Kenya. Values for plankton and mud are means of three replicates. DAP (diammonium phosphate) and urea were used as chemical fertilizers. Rice bran served as an organic fertilizer and a supplemental feed. See text for full explanation of treatments.

Treatment (TX) Number and Description	$\delta^{13}C$ ± S.D. (‰)			
	Oreochromis	*Clarias*	*Plankton*	*Mud*
1 DAP (4 kg P ha^{-1} wk^{-1}) + Urea (16 kg N ha^{-1} wk^{-1})	-16.6 ± 0.7[c]	-19.8 ± 1.2[c]	-17.3 ± 0.5[a]	-13.5 ± 1.4
2 ½ (DAP + Urea) of TX 1 + ½ Rice Bran of TX 3	-16.6 ± 0.7[c]	-19.8 ± 1.2[c]	-23.5 ± 1.6[b]	-13.1 ± 0.6
3 Rice Bran (120 kg ha^{-1} d^{-1})	-16.6 ± 0.7[c]	-19.8 ± 1.2[c]	-26.3 ± 2.5[b]	-13.3 ± 0.7
4 Rice Bran of TX 3 + ½ (DAP + Urea) of TX 1	-16.6 ± 0.7[c]	-19.8 ± 1.2[c]	-26.8 ± 2.5[b]	-12.3 ± 0.5

[a,b] Means in columns with different letters are significantly different ($P < 0.05$) according to Fisher's Least Significant Difference test.
[c] Initial isotope values for *Oreochromis* and *Clarias* are means of 5 individual fish taken from a group of fish fed a single conditioning diet in a common pond prior to stocking in individual ponds.

Table 2. Midpoint stable carbon isotope values ($\delta^{13}C$) of pond components in a feeding trial with *Oreochromis* and *Clarias* in Sagana, Kenya. Values are means of three replicates. DAP (diammonium phosphate) and urea were used as chemical fertilizers. Rice bran served as an organic fertilizer and a supplemental feed. See text for full explanation of treatments.

Treatment (TX) Number and Description	$\delta^{13}C$ ± S.D. (‰)			
	Oreochromis	*Clarias*	*Plankton*	*Mud*
1 DAP (4 kg P ha^{-1} wk^{-1}) + Urea (16 kg N ha^{-1} wk^{-1})	-17.9 ± 0.9[a]	-22.4 ± 2.1[a]	-22.4 ± 1.8[a]	-13.7 ± 1.1
2 ½ (DAP + Urea) of TX 1 + ½ Rice Bran of TX 3	-22.8 ± 0.7[b]	-24.7 ± 1.3[ab]	-27.0 ± 1.2[b]	-14.6 ± 1.1
3 Rice Bran (120 kg ha^{-1} d^{-1})	-22.9 ± 2.4[b]	-26.4 ± 1.2[b]	-29.6 ± 1.2[b]	-14.7 ± 0.9
4 Rice Bran of TX 3 + ½ (DAP + Urea) of TX 1	-24.0 ± 1.2[b]	-26.8 ± 0.6[b]	-27.8 ± 1.8[b]	-15.4 ± 0.9

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

Table 3. Final stable carbon isotope values ($\delta^{13}C$) of pond components in a feeding trial with *Oreochromis* and *Clarias* in Sagana, Kenya. Values are means of three replicates. DAP (diammonium phosphate) and urea were used as chemical fertilizers. Rice bran served as an organic fertilizer and a supplemental feed. See text for full explanation of treatments.

Treatment (TX) Number and Description	$\delta^{13}C$ ± S.D. (‰)			
	Oreochromis	*Clarias*	*Plankton*	*Mud*
1 DAP (4 kg P ha^{-1} wk^{-1}) + Urea (16 kg N ha^{-1} wk^{-1})	-17.9 ± 0.7[a]	-18.5 ± 1.1[a]	-20.6 ± 3.4[a]	-14.0 ± 0.5
2 ½ (DAP + Urea) of TX 1 + ½ Rice Bran of TX 3	-23.7 ± 1.2[b]	-24.7 ± 0.2[b]	-25.7 ± 1.0[b]	-16.2 ± 1.8
3 Rice Bran (120 kg ha^{-1} d^{-1})	-25.8 ± 0.1[c]	-27.2 ± 0.9[c]	-29.3 ± 0.5[b]	-16.1 ± 1.1
4 Rice Bran of TX 3 + ½ (DAP + Urea) of TX 1	-24.9 ± 0.6[bc]	-25.9 ± 1.1[bc]	-26.0 ± 1.5[b]	-15.6 ± 0.6

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

The mean isotope ratios of *Oreochromis* became substantially more negative between the initial and midpoint sampling periods for treatments 2, 3, and 4, and slightly more negative between the midpoint and final sampling periods (Tables 1 through 3). However, the mean $\delta^{13}C$ of *Oreochromis* in treatment 1 changed very little during the study (Tables 1 through 3). The mean isotope ratios of *Clarias* became more negative between the initial and midpoint sampling periods for all treatments (Tables 1 and 2). The mean isotope ratios of *Clarias* changed only slightly between the midpoint and final sampling periods in treatments 2, 3, and 4 (Tables 2 and 3), while that of *Clarias* in treatment 1 became about 4‰ more positive (Tables 2 and 3). The mean isotope ratios of plankton became more negative in all treatments between the initial and midpoint sampling periods (Tables 1 and 2), then more positive between the midpoint and final periods (Tables 2 and 3). However, the magnitude of the change in plankton isotope ratios within treatments was small (approximately 3‰) for the whole study period. The mean isotope ratios of mud became more negative for all treatments throughout the study (Tables 1 through 3), although the changes were more pronounced for treatments 2, 3, and 4 than for treatment 1. As with the plankton, the magnitude of the changes in mean isotope ratios of mud within treatments was small (3‰ or less) for the whole study period.

DISCUSSION

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). Several months prior to collection of initial samples, *Oreochromis* and *Clarias* were fed a conditioning diet containing corn to increase the isotopic resemblance of the fish to corn (-14‰). Corn is isotopically distinct from the rice bran (-27.8‰) used as a supplemental feed in treatments 2, 3, and 4. The isotope ratios of the *Oreochromis* and *Clarias* before receiving the corn diet were unknown so the influence of the corn diet on the isotope ratios of fish at the initiation of the study is not certain. Although the isotope ratios of initial *Oreochromis* (-16.6‰) and *Clarias* (-19.8‰) were 3 to 6‰ different from that of corn, they were 8‰ or more different from that of rice bran.

The most distinct trend in the isotope data was the more positive values for plankton, *Oreochromis*, and *Clarias* in treatment 1 versus treatments 2 through 4 for initial, midpoint, and final samples. Treatment 1 did not include rice bran, whereas treatments 2 through 4 did. Gut content data of Veverica et al. (2000) indicated that both *Oreochromis* and *Clarias* in treatments 2 through 4 consumed rice bran directly, which contributed to the more negative isotope values of these fish compared to those in treatment 1. However, the isotope values of the plankton in treatment 1 were more positive initially than those of the plankton in the other treatments, which suggests that the result may be due to an undefined pretreatment effect. Veverica also observed significantly higher chlorophyll *a* concentrations, as well as lower weight gain of *Oreochromis* and *Clarias* in treatment 1 compared to the other treatments (Veverica et al., 2000).

The addition of rice bran to ponds in treatments 2 through 4 clearly increased fish weight gain relative to ponds where the only inputs were DAP and urea (Veverica et al., 2000). Gut content analysis (Veverica et al., 2000) indicated that the two most important food categories for *Oreochromis* and *Clarias* in treatments 2 through 4 were plankton and rice bran. The isotope data did not allow further clarification of the relative nutritional importance of the two categories between treatments because the isotope ratios of plankton and rice bran differed by only 4‰ or less. The DAP (-26.8‰) was also isotopically similar to rice bran (-27.8‰), but DAP did not appear to have much influence on the isotope value of the plankton in treatment 1 (no rice bran).

The discriminating power of the isotope technique may be improved in future studies by fractionating the plankton into different categories before isotope analysis, as fish gut content analysis indicated that there were major differences in the relative use of zooplankton and phytoplankton for *Oreochromis* and *Clarias* in the different treatments. The effect of the different fertilization regimes on primary and secondary plankton production rates would be useful in optimizing nutrient utilization of co-cultured fish species with different trophic habits. In addition, the use of nitrogen isotope data might clarify the trophic relationships between *Oreochromis* and *Clarias* in different treatments. Gut content data were not useful in this respect, as *Oreochromis* guts contained an equal or higher number of fish scales than *Clarias* guts in treatments 2 through 4.

ANTICIPATED BENEFITS

Production efficiency of *Oreochromis* and *Clarias* can be optimized once the quantitative importance of different nutrients under defined experimental conditions is established using the isotope technique (perhaps multiple isotope tracers) 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.

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